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Procedures for Measuring Cough (Gill Purge) Rates of Fish

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PROCEDURES FOR MEASURING COUGH (GILL PURGE) RATES OF FISH

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FOREWORD

When this manuscript appears in print, twelve months will have elapsed since the passage of the Toxic Substances Control Act by the United States Congress. Never before in the history of any large industrial country has there been a requirement to so extensively test and evaluate all chemicals before they are produced and marketed. This is a truly monumental task, particularly as we must evaluate not only the thousands of chemicals currently in use, but new ones produced annually.

This report outlines results of nearly ten years of research at the Environmental Research Laboratory-Duluth. For years, toxicologists have sought short term methods to predict long term effects of chemicals. The gill purge response described in this report has a better correlation between the endpoints observed and the no-effect concentrations measured for a variety of toxic chemicals than any other approach currently available. Furthermore, it is quick and relatively inexpensive to perform. The opinion of our laboratory is that this test has had adequate testing and that there is a sufficient data base upon which to conclude that it is one of the best measurements to predict the long term effects of toxic materials yet devised. We further believe that this test has the best biomonitoring potential for use directly on waste streams of any biomonitoring technique yet proposed. We think that this technique is ready to be used on a wide scale both in screening toxic materials for the Toxic Substances Control Act, and for monitoring complex and simple effluents from current discharges.

From this publication regulatory agencies ought to be able to develop precise test protocol for unique and specific application.

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Environmental Research Laboratory
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ABSTRACT

The cough (gill purge) is an interruption in the normal ventilatory cycle of fish that serves to clean the gills of accumulated particulate matter. A review of the literature shows that the cough occurs in a variety of freshwater and marine fish; that both mechanical and chemical stimulation apparently can cause fish to increase their cough rates; and that an increase in coughing is a rapid and sensitive endpoint for studying chemicals and effluents. In reviewing the test methods and apparatus for measuring cough rates of fish, we conclude the electrode chamber method offers more potential as a bioassay tool for assessing the respiratory responses of fish due to toxicant exposure. Recommended test procedures, based on our experience, for using the electrode chamber method are given.

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SECTION 1

INTRODUCTION

Various behavioral and physiological aberrations of fish have been used over the years to indicate stress due to the presence of toxic materials. Changes in cough frequency, opercular rate, respiration rate (oxygen consumption), feeding patterns, locomotor activity, fin movements, eye movements, reproductive displays, swimming ability, avoidance, and the ability to respond to a stimulus (conditioned response) all have been used as a measure of such stress. Of these, respiratory impairment in fish is a sensitive indicator of toxicant stress, but coughing rate shows more promise as a significant response to sublethal concentrations than opercular rate (Sprague 1971). We believe that change in cough frequency has wide potential application as a bioassay tool because (1) the response occurs in a variety of fish, (2) many chemical substances are known to elicit the response, (3) the endpoint is rapid and sensitive, (4) the degree of response usually occurs in direct proportion to the toxicant concentration, (5) the cough is predictive of long-term adverse effects at levels near the maximum acceptable toxicant concentration (MATC) for most of the single toxicants investigated to date, and (6) an organism can act as an integrating sensor of potentially toxic conditions when exposed to complex effluents.

Although fish coughs have been recognized for many years and a considerable amount of effort has been expended on examining this phenomenon, no comprehensive review of the fish-cough literature is presently available. One purpose of this paper is to fulfill this need. Also, we have found that some procedures are better than others for detecting and recording the cough and other movements of fish. The main purpose of this paper is to describe these procedures and discuss the state-of-the-art as we see it. This discussion will include observations we have made that remain unpublished as well as those we and others have published. Although this report is written primarily for those who do not know how such tests can best be conducted and how the data can be applied, we hope that it will also contain useful information for those presently using increases in cough rate as a test endpoint.

SECTION 2

CONCLUSIONS

1. The cough is an interruption in the normal ventilatory cycle of fish, which in ordinary water serves to clean the gills of accumulated particulate matter. When particulate content of the water is increased, cough rates increase accordingly. Increases in cough rate also can be elicited by exposing fish to chemicals and effluents devoid of particulate matter. Thus, both mechanical and chemical stimulation can cause fish to increase their cough rates.

2. A review of the literature shows that the cough occurs in a variety of freshwater and marine fish; that, as a test endpoint, it is rapid and sensitive; that the response occurs at sublethal levels of toxicants, and usually in direct proportion to toxicant concentration; and that it is predictive of long-term adverse effects on fish at levels near the maximum acceptable toxicant concentration (MATC) for most of those single toxicants tested to date.

3. Respiratory movements of fish, including coughs, can be detected and recorded by use of pressure transducers that measure water-pressure changes within the buccal or opercular cavities of a cannulated fish, or by electrode chambers that use external, unimplanted electrodes to detect the action potentials associated with the respiratory movements of a free-swimming fish. Although the pressure transducer method has yielded considerable information about the respiratory events that occur in fish, the electrode chamber method offers more potential as a bioassay tool for assessing the respiratory responses of fish due to toxicant exposure.

SECTION 3

RECOMMENDATIONS

1. We recommend that studies be initiated to determine why fish respond to various chemical substances by increasing the rate at which they cough. The mode of action needs to be established according to the type of chemical to which the fish are exposed.

2. The influence of physical factors such as salinity, conductivity, temperature, and pH on cough rates needs further study.

3. More comparative studies are needed to determine if concentrations known to cause long-term adverse effects also cause significant increases in cough rates of fish tested under similar experimental conditions. Correlations of this type are required to assess the utility of using increases in coughing as a predictor of long-term (chronic) effects.

4. Computer programs should be developed that are capable of distinguishing coughs from other biological activities, tabulating these data, and statistically analyzing the results.

5. Methods for statistically analyzing and graphically interpreting cough-rate data should be standardized.

6. We recommend that in the future fish "coughs" be referred to as "gill purges" and that the purge rate be expressed as gill purges per minute. Gill purge describes the physiological event more accurately than terms such as cough, cough reflex, gill clearing or cleaning, etc.

SECTION 4

REVIEW OF LITERATURE

PHYSIOLOGICAL STUDIES

In ordinary water fish periodically rid the gill surfaces of accumulated foreign material by a gill-clearing action referred to as a "cough" or "cough reflex". Fish coughs have been observed and studied for many years beginning with Bert (1870), Francois-Franck (1906), Kuiper (1907), and Bijtel (1949). Coughs have been observed in freshwater and marine teleosts (Hughes and Shelton 1958; Hughes 1960), elasmobranchs (Satchell and Maddalena 1972) and lampreys (Rovainen 1977). Neurological events associated with coughing have also been studied (Shelton 1959; Young 1970, 1972; Ballintijn and Roberts 1976).

So far as we know, there are two basic types of coughs. A small, or weak, cough consists of a greater than normal expansion and contraction of the buccal and opercular cavities, particularly the buccal. This action produces a higher than normal positive pressure in the buccal cavity and results in a forceful movement of water across the gills in the normal direction. This pulse may be preceded by a momentary reversal of water flow over the gills (Holeton and Jones 1975). A large, or strong, cough occurs whenever the pressure gradient between the buccal and opercular cavities is reversed. In this case the buccal cavity develops a high, uncharacteristic negative pressure relative to the opercular cavity, and as a result water flow reverses across the gills. During the reversal debris or mucus is ejected rapidly through the mouth. The reversal phase is quickly followed by a prolonged, high positive pressure in the buccal cavity, after which normal pressure cycles are resumed. The physiological basis of the cough is well documented, and important findings have been summarized by Hughes (1973), Hughes and Morgan (1973), Holeton and Jones (1975), Hughes (1975), and Hughes and Adeney (1977).

METHODS FOR DETECTING COUGHS

Over the years a number of researchers have counted fish coughs by observing the fish directly. This method is still used by a few investigators (McPherson 1973; Bull and McInerney 1974; Hargis 1976), but most researchers have found it advantageous to record coughs at an instrument station distant from the test area. One approach is to record the water-pressure changes that take place in the buccal and opercular cavities of the fish. A tube is placed adjacent to these cavities (Hughes and Shelton 1958) or a cannula is inserted directly into the fish (Saunders 1961), and changes in pressure are measured by a pressure transducer. The resulting signals are then displayed on an oscilloscope or recorded on a polygraph strip-chart recorder. Several

researchers have used this method with slight modifications (Ballintijn and Hughes 1965; Schaumburg, Howard, and Walden 1967; Hughes and Saunders 1970; Hughes and Roberts 1970; Young 1972; Sparks, Cairns, McNabb, and Sutter 1972; Davis 1973; Sellers, Heath, and Bass 1975; Bimber, Boenig, and Sharma 1976).

Another approach differs from the above in that action potentials (bioelectric potentials) associated with the animal's movements (Camougis 1960; Goodman and Weinberger 1971; Heath 1972) are measured instead of pressure changes. Electrodes, usually stainless steel wire, are placed at each end of an electrode chamber containing one free-swimming fish. Electrical signals in the microvolt range emanating from the fish are picked up by the electrodes and transmitted via shielded cable to high-gain preamplifiers. The amplified signals are then displayed on a strip-chart recorder (Spoor, Neiheisel, and Drummond 1971; Drummond, Spoor, and Olson 1973; Drummond, Olson, and Batterman 1974).

RESPONSE TO PHYSICAL AND CHEMICAL SUBSTANCES

In addition to the debris and other foreign materials found in ordinary water (Heimstra, Damkot, and Benson 1969), fish respond to other physical and chemical substances by changing the rate at which they cough. In general, cough frequency increases as the concentration of these substances increases, but the mode of action is not well understood. Schaumburg et al. (1967) suggested that increases in coughing might be due to the lowering of the dissolved oxygen at the gill surface. Hughes (1975) felt that coughing is mainly a response to some form of mechanical stimulation, the precise nature of which is unknown, but Davis (1973) concluded that the response was due to chemical as well as mechanical stimulation. Substances known to affect the cough rates of fish are grouped in four categories: metals, pesticides, effluents, and miscellaneous.

Metals

Drummond et al. (1973) reported that cough frequency in brook trout (Salvelinus fontinalis) increased significantly at a copper concentration as low as 0.009 mg/l and that cough rate increased proportionately as the concentration was increased. Because 0.009 mg/l was just below the maximum acceptable toxicant concentration (MATC, described by Mount and Stephan 1967) determined by McKim and Benoit (1971), the response was considered predictive of the concentration range of copper likely to have no long-term adverse effects on the species. Cough frequencies of sockeye salmon (Onchorhynchus nerka) increased significantly at all copper concentrations above 0.024 mg/l (Martens, Gordon, and Servizi 1970). Increases in coughing were noted among rainbow trout (Salmo gairdneri) and bluegill sunfish (Lepomis macrochirus) exposed to zinc (Skidmore 1970; Sparks et al. 1972; Hughes 1975). Sprague (1964) observed that young Atlantic salmon (Salmo salar) coughed whenever they swam into water containing a high concentration of copper or zinc. The effect of mixtures of copper and zinc on the cough rate of rainbow trout has also been studied (Sellers et al. 1975). Bluegill sunfish showed significant increases in cough frequency at cadmium concentrations of 0.050, 0.084, and

0.115 mg/1, and the increases were proportional to the concentrations (McIntosh and Bishop 1976).

McPherson (1973) found a linear relationship between the concentration of methylmercuric chloride (MMC) and the number of cough reflexes per 100 opercular movements when testing rainbow trout and coho salmon (Oncorhynchus kisutch). The lowest concentration of MMC and mercuric chloride to cause a significant increase in cough frequency in brook trout was reported by Drummond et al. (1974) to be 0.003 mg/1. Because there were no discernible adverse effects to the first generation of brook trout at concentrations below 0.0029 mg/1 MMC during a three-generation exposure (McKim, Olson, Holcombe, and Hunt 1976), the cough response was determined to be a short-term indicator of the long-term effects of MMC to that species. Cough frequency also increased in direct proportion to MMC concentrations tested above 0.003 mg/1.

Pesticides

A direct relationship was demonstrated between the concentration of DDT and the cough rate of coho salmon (Schaumburg et al. 1967); a significant increase in cough rate occurred at as low a concentration as 0.005 mg/1. Bull and McInerney (1974) examined several behavioral endpoints of coho salmon and found that only coughing increased in response to increasing concentrations of the insecticide Fenitrothion. Rainbow trout exposed to DDT, dieldrin, and carbaryl (a methyl carbamate insecticide) showed increases in cough frequency, and, except for carbaryl, the increases were proportional to toxicant concentration (Lunn, Toews, and Pree 1976). Concentrations of 1.0 and 5.0 mg/1 diaquat caused yellow perch (Perca flavescens) to increase their cough frequency significantly; the response was greater at the higher concentration (Bimber et al. 1976).

Effluents

With the exception of kraft pulp-mill effluents (KPME) little work has been done in which fish coughs are used as an indicator of effluent toxicity. Walden, Howard, and Froud (1970) conducted several experiments with KPME collected from two different pulp mills. They found dramatic increases in cough frequency of rainbow trout exposed to high concentrations of effluent. As effluent concentration approached lethal levels, the cough rate rose steadily, became erratic, and could not be correlated with the concentration. But at lesser effluent concentrations cough frequency increased, reached a maximum, and then declined to the normal pre-exposure rate. The maximum increases in cough frequency at these lesser concentrations were directly proportional to the effluent concentration.

Davis (1973) exposed sockeye salmon to KPME and found that increases in cough rate began at 20 percent of the 96-hr LC50. He noted that other workers reported reduced specific growth rates for this species at the same concentration. Howard and Walden (1974) stated that the cough reflex is one of several biological indicators that show promise for monitoring the effectiveness of treatment systems designed to upgrade the quality of pulp-mill effluents. They showed that rainbow trout exposed to an untreated KPME increased their cough frequency significantly, but when exposed to the same

effluent after proper treatment, cough frequencies were not significantly different from those of control fish. In a more recent study Carlson and Drummond (1977) demonstrated that increases in bluegill sunfish cough rate were commensurate with effluent concentration when the fish were exposed to a variety of treated industrial and municipal effluents. We concluded that cough frequency is a rapid and sensitive physiological characteristic for evaluating the relative quality of treated effluents and variations in quality of same-source effluents, before discharge into receiving waters.

Miscellaneous

Brook trout increased their cough frequency when exposed to chlorine and sulfides (Dandy 1967, 1972), and rainbow trout exposed to chlorine showed a similar response (Bass 1975). Servizi, Gordon, and Martens (1969) noted that sockeye salmon smolts showed increases in coughing at initial hydrogen sulfide concentrations between 0.3 and 1.1 mg/1. Ammonia, alkylbenzene sulfonate, and coal dust were reported to cause increases in fish-cough frequency (Hughes 1975). MacLeod and Smith (1966) found that fathead minnows (Pimephales promelas) increased their cough rate relative to fiber concentration when exposed to suspension of pulpwood fibers. Water-soluble fractions of oils caused pink salmon fry (Oncorhynchus gorbuscha) to increase their cough rate in proportion to concentrations $\geq$ 30% of the 96 hr median tolerance limit (Rice, Thomas, and Short 1977).

Water temperature and pH reportedly influence the rate at which fish cough. While studying the respiratory pumps of rainbow trout, Hughes and Roberts (1970) noted an increase in the number of reversals and double reversals in respiratory pressure (presumably coughs) as the water temperature was raised. Rainbow trout tested at pH 6 showed significantly fewer coughs than those treated at pH 9 (Hargis 1976), although cough rates of brook trout tested at pH 6 and 9 were not significantly different according to Drummond et al. (1974).

SECTION 5

RECOMMENDED TEST METHOD

In comparing the methods for measuring fish respiratory movements Heath (1972) concluded that observing fish directly had several disadvantages as a test method. Although the procedure does have some disadvantages, it can be a useful technique to provide first-hand observations that could not be obtained by other means. Heath (1972) further concluded that recording respiratory pressures with cannulated fish is a method that yields the most information with the least effect on the fish. Detection or measurement of coughing is mentioned only in conjuction with indwelling cannulae, even though he noted a one-to-one correspondence between opercular movements recorded simultaneously by using cannulae and external electrodes (electrode chambers). Heath's comments seem to imply that the electrode chamber method is suitable for recording opercular movements, but that the cannulae/pressure-transducer method should be used to record cough, opercular and other respiratory movements. We compared the two methods and believe that the electrode chamber method, when used with the proper instrumentation, is the better technique of the two for a number of reasons.

We simultaneously recorded coughs and opercular movements of bluegill sunfish by using the cannulation (Saunders 1961) and the electrode chamber (Spoor et al. 1971) methods. We found that coughs can be distinguished from the more rhythmic opercular waveforms with either method and that a one-to-one correspondence exists between coughs and opercular movements recorded simultaneously as action potentials and water-pressure changes (Figure 1). The recordings of the coughs and opercular movements obtained simultaneously by the two methods also were verified by observing some of the fish and correlating our observations with what appeared on the strip-chart records. In addition to coughs and opercular movements, both methods enabled us to detect and record changes in opercular amplitude, yawns (McCutcheon 1970), and body movements (Appendix C). The electrode chamber method also enabled us to detect and record fin movements, muscle spasms, and, in some instances, the heart beat of trout. Although these signals are usually masked by the stronger action potentials associated with cough, opercular, and body movements, some of the weaker signals can be separated from the other signals by electronic filters (Venables and Smith 1972; Rommel 1973; Strange, Dean, Dorman, and Fletcher 1975).

The electrode chamber method has a big advantage over the cannulae/pressure-transducer method in that it permits study of free-swimming fish that have not suffered the trauma of anesthetization and operative procedures. We have observed that cannulated fish never fully recover (i.e., cough and opercular rates remain higher than normal) from the effects of surgery and having a

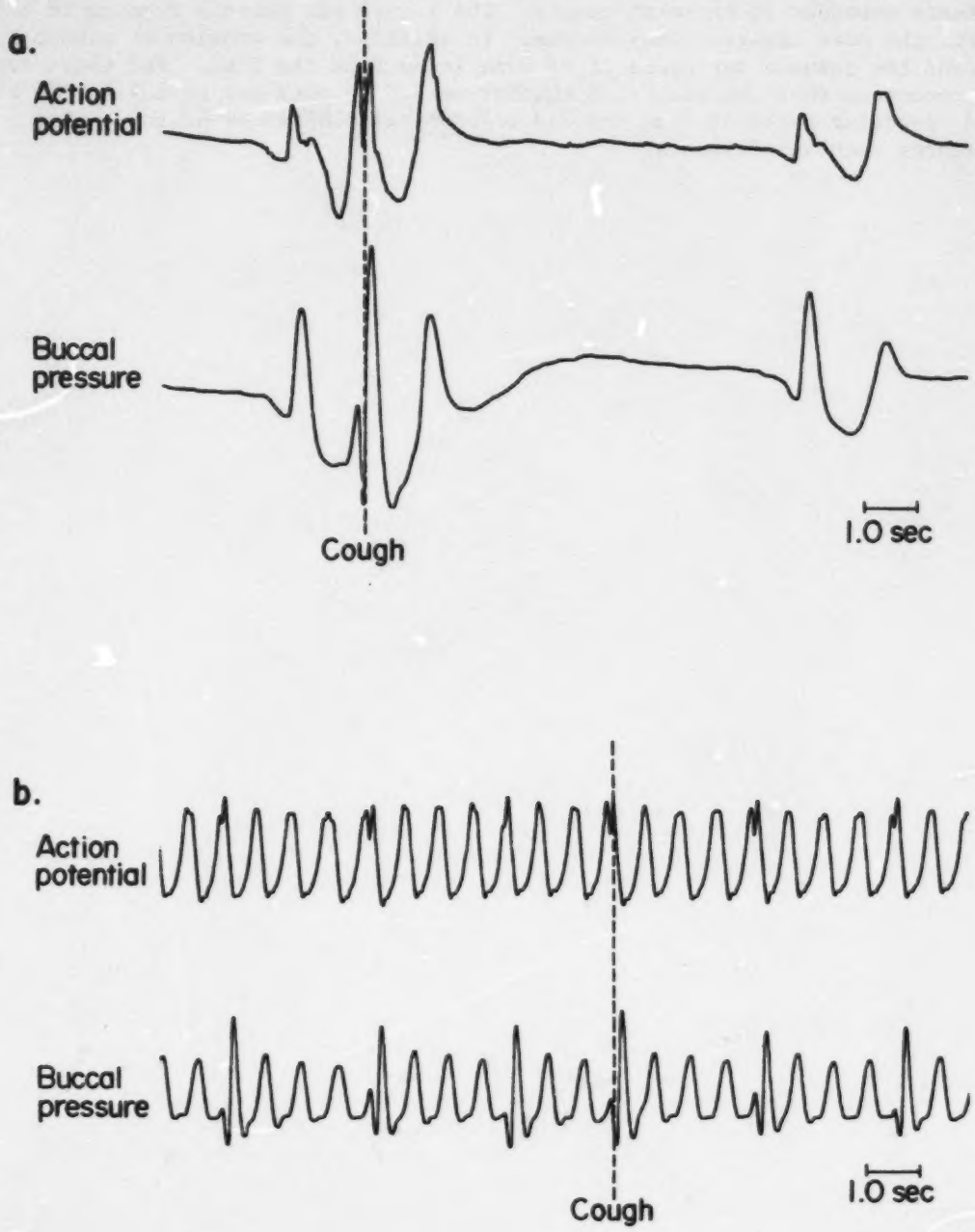


Figure 1. Respiratory patterns of bluegill sunfish recorded simultaneously by the pressure-transducer and electrode chamber method while a) in control water, and b) undergoing toxicant stress. Note presence of other coughs (unmarked).

cannula imbedded in the oral cavity. The longer the cannula remains in the fish, the more stressed they become. In addition, the erosion of tissue around the cannula may cause it to come loose from the fish. For these reasons we recommend that the electrode chamber method be used for measuring the cough and opercular rates of fish exposed to chemical substances or to complex mixtures such as effluents.

SECTION 6

RECOMMENDED TEST APPARATUS

The basic system consists of electrode chambers, amplifiers, and servo-pens to record the various action potentials produced by the fish on strip-chart records (Figure 2). The easiest way to set up this system is to build the chambers yourself and purchase a commercial polygraph (2-12 channels are available) consisting of preamplifiers, amplifiers, servo-pens, and a strip-chart drive. A commercial polygraph costs about one thousand dollars per channel, but cost of the instrumentation can be reduced by using a switching device so that one channel can be used to monitor more than one fish (Figure 2). In the following detailed description of the system we will emphasize those considerations that we believe are important.

CHAMBERS

Basic Design

The basic design of the electrode chamber has been given by Spoor et al. (1971). Although the chambers can be modified to fit a particular need, the concept remains unchanged. The chambers should be rectangular and narrow (roughly, not more than 3x the width of a fish) so that the fish is forced to assume a position perpendicular to the ends of the chamber, i.e., facing one of the two electrodes. If the chambers are too wide, the fish can assume a position off to one side of the chamber or parallel to the electrodes; this results in considerable loss of signal amplitude. For toxicity tests the chambers should be made of glass cemented together with a clear silicone adhesive. A horizontal, cylindrical chamber may be required for studying some fish species such as fathead minnows (Drummond 1977).

Multiple Chamber Units

Chambers can be put together either individually or in banks of two, four, six, etc. Each fish is still monitored individually whether the chambers are constructed as single or multiple units. It is convenient to have the same number of chambers and recording channels in each bank; for example, a bank of four chambers is best used with a four-channel polygraph. Also, working with and cleaning four units of four chambers is easier than working with 16 individual chambers. We have described individual chambers, but these designs can be expanded readily into multiple units.

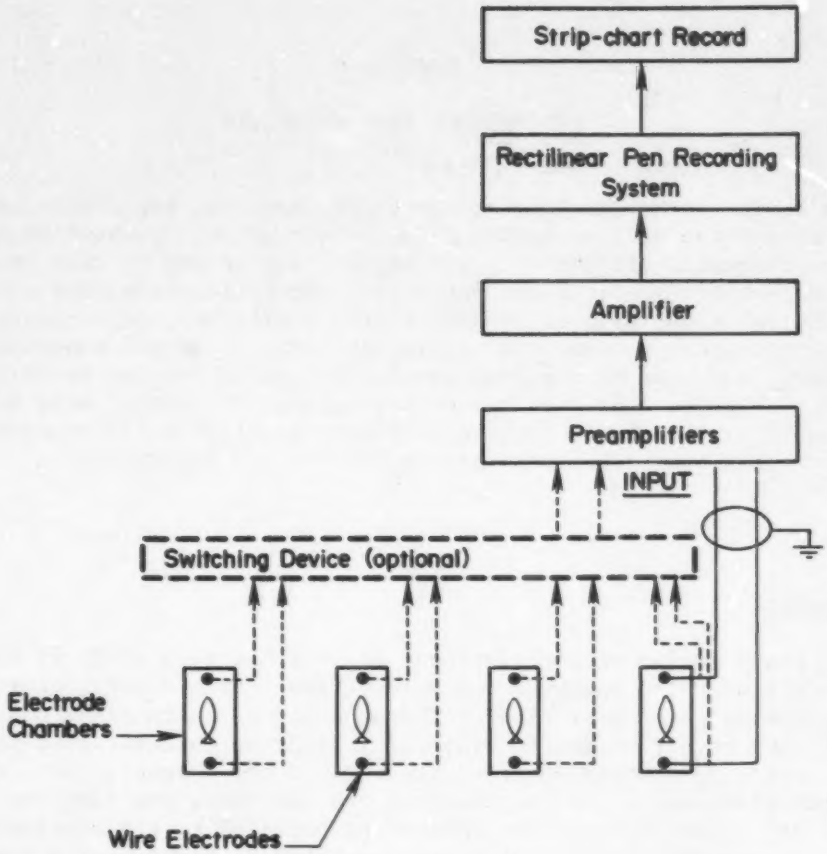


Figure 2. Schematic of the electrode chamber system.

Flow-through Chambers

Incoming water normally enters one end of the chamber, passes through the chamber, and exits through a drain at the other end. Constant level headboxes, fitted with capillary tubes or syringe needles, are the most convenient and reliable method of water delivery (Figure 4, Section 7). Chambers can be drained by a glass standpipe or by a hole, fitted with a stopper and drain tube, in the endplate.

Water can also be delivered to a chamber by air-lifting it up from a tray placed immediately below the chamber. The air-lifted water passes through the chamber, returning to the tray via a standpipe drain (Appendix A). Although this system can be used for flow-through testing, it was designed to be used as a recirculating test system and is particularly useful for testing imported samples of effluents. A detailed description of this design, based on an actual system used for testing effluent samples, is given in Appendix A.

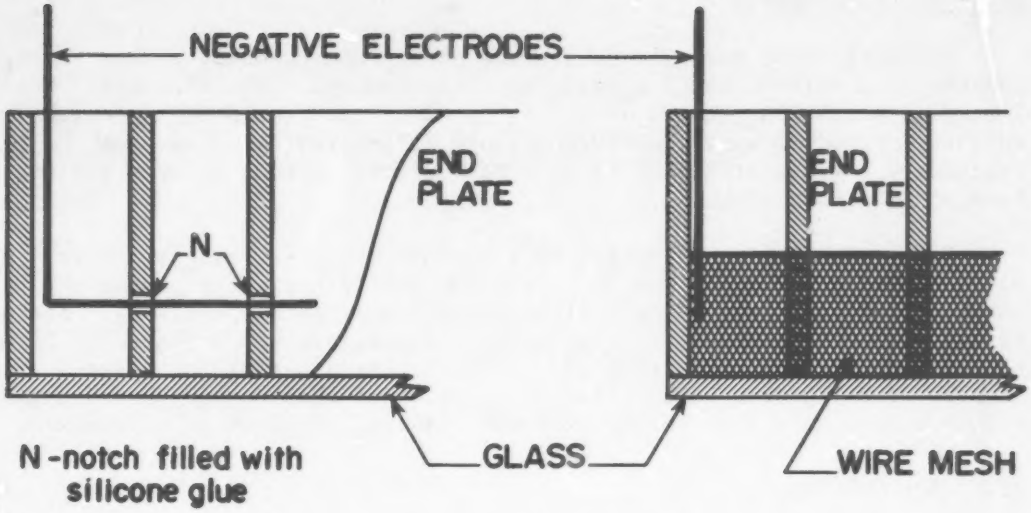
Immersed Chambers

Chambers designed to be immersed in a tank (aquarium) of water are suitable for both flow-through and recirculating tests (Appendix B). In the flow-through mode this design is especially useful for on-site monitoring of effluents because separate flows to each chamber (multiple chamber design) are not required. As with the airlift chambers, they can be used in the flow-through mode during the acclimation period and, when the test begins, can be converted to the recirculating mode. Immersed chambers require wire-mesh electrodes at each end to keep the fish inside the chamber and permit circulation of water through the chamber. Recirculation is very important because without it, fish quickly deplete the dissolved oxygen content of the water inside the chamber. Aeration is the easiest way to promote necessary circulation. Details of a four-plex, immersed chamber design used in some of our effluent tests are given in Appendix B.

Placement of Electrodes

Stainless steel wire or mesh electrodes are glued with silicone to the endplates of the chamber. The distance between the electrodes should not exceed two times the length of the fish. For versality, one electrode can be glued to the endplate and the other to a moveable glass baffle (Appendix A). This arrangement permits increasing or decreasing the interior length of the chamber to accomodate fish of different sizes. Unless mesh electrodes are required to keep the fish inside immersed chambers, wire electrodes (1.5 mm diameter) work just as well as mesh electrodes.

Each pair of chamber electrodes consists of a positive and a negative electrode. Although electrodes are usually arranged in pairs (Figure 2), this arrangement need not be used when several chambers are put together as a unit. In this case the positive electrodes are placed at the inlets and a common negative electrode is placed at the outlet of the chambers. Two examples of how the negative electrode can be attached to the chambers follow:



One word of caution. This arrangement (Figure 3) worked well with a Narco physiograph, but did not work with a four-channel, homemade amplifier based on the design given by Drummond and Dawson (1974). The problem seemed to be in the circuitry in that a single input was recorded on all four channels. The choice of using pairs of independent electrodes (Figure 2) or individual positive electrodes and a common negative electrode (Figure 3) may depend on the type of polygraph used. We suggest testing both types of electrode arrangements to determine which one works best.

Electrodes should be connected to the amplifiers with shielded leads. In addition to shielded leads, the water supply (headboxes) and ungrounded equipment in the vicinity of the test area should be grounded. All ground wires should be connected to the same ground potential. Larger microphone cables work well with the pin jack/electrode connection described by Spoor et al. (1971). Smaller diameter leads, such as Belden shielded instrumentation cable, are convenient to work with because of their size, but tend to break after being repeatedly connected and disconnected when pin jack connectors are used. To avoid this problem we permanently attached the shielded leads to male pin terminals, in a junction box, and connected the electrodes to these terminals with patch cords (Figure 3). Patch cords, having female terminals at each end, can be made up from a kit (Waldom/Molex Designer Kit No. WM-72); they are easy to connect and disconnect from the electrodes, and they are durable.

RECORDING SYSTEM

A number of commercial polygraphs are suitable for detecting and recording coughs and other movements of fish. Although we cannot recommend one brand over another, we can outline those considerations we believe to be important.

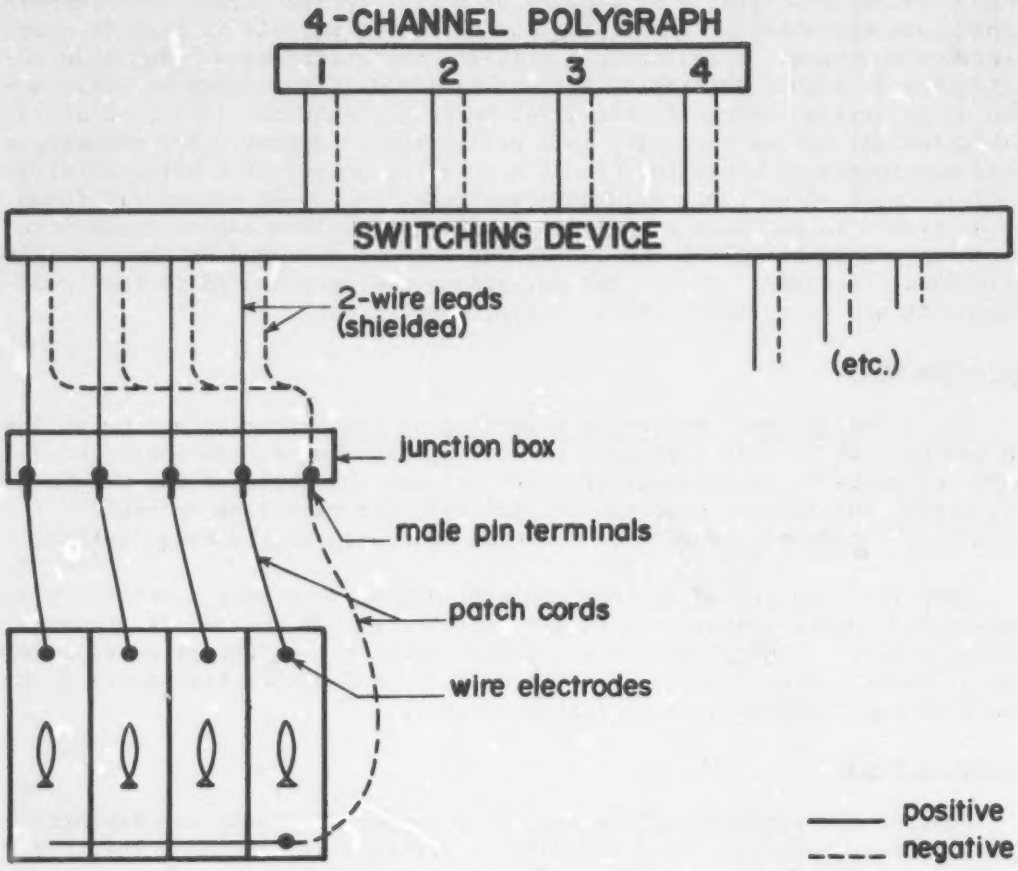


Figure 3. Wiring schematic for multiple chamber units.

Amplifiers

Input signals in the low microvolt range require two stages of amplification (preamplifiers and amplifiers) to amplify them into the millivolt range. Polygraphs capable of amplifying human electroencephalogram input signals are suitable for amplifying the action potentials of fish studied in electrode chambers. Solid-state amplifiers are recommended. Built-in 60-Hz filtration is highly desirable, but can be added if necessary by using some type of filtering device (Ferris 1974; Ewing and Ashworth 1974). Elaborate filtration may not be necessary in a well-grounded system. For example, a 5-mfd non-polarized capacitor placed across the output of a Narco physiograph amplifier will effectively eliminate any remaining 60-Hz noise. If funds are not available to purchase a commercial unit, amplifiers can be constructed (Drummond and Dawson 1974) and the output signals recorded on any rectilinear, strip-chart recorder. Commercial polygraphs are recommended in that all components are integrated into a single, compact unit.

Recording Pens

Use a rectilinear, servo-pen recording system rather than a curvilinear pen system. It is very difficult to distinguish coughs from opercular and other movements on curvilinear tracings because the shape of the waveform is distorted. Curvilinear tracings are suitable for recording opercular movements, but do not provide a clear-cut recording of the cough reflex.

Many rectilinear pen systems are available. Some have a capillary or pressurized inking system; others are equipped with a thermal or pressure writing system. Inking systems are quite reliable even though messy to work with at times. Heated pens also work well if used with a high quality, heat-sensitive paper coated with a plastic material.

Recording Paper

Cost of the recording paper must be considered. Ewing and Ashworth (1974) stated that electrical recording paper is more expensive than thermal paper, and both types are more costly than paper suitable for ink writing. Both sides of inking paper, at least the Z-fold type, can be used to further reduce the cost. Since monitoring is usually done quite frequently, paper should be long enough to permit extended monitoring without changing paper. Z-fold packets or rolls 152 m (500 ft) long are recommended. For economy the paper should be run at the slowest possible speed, yet fast enough so that the coughs can be seen clearly and counted. We found 0.1 cm/sec to be an optimum paper speed for monitoring most fish species, but faster speeds (0.25-2.5 cm/sec) should be available for use as the need arises.

Automatic Data Processing

Although strip-charts provide a permanent record of the fish's movements, counts and other measurements must be made manually. This can become a tedious and time-consuming task in a large-scale testing program. An alternative is to feed the amplified signals into some type of automatic data-processing unit (Cairns et al. 1973); Morgan and Kuhn 1974; Dessy 1976;

Morgan 1976, 1977). As far as we know, coughs have not been separated from the other signals with this type of instrumentation. The development of a software program should not be too difficult, however, that will separate coughs from other fish movements and automatically tabulate cough rates before and after fish are exposed to chemical substances or effluents.

SECTION 7

RECOMMENDED TEST PROCEDURES

Since many procedures apply to toxicity testing in general, e.g., treatments for diseased fish, we will describe only those procedures closely allied to the electrode chamber method. Other appropriate references should be consulted before cough-response tests are started (U.S. Environmental Protection Agency 1975; American Public Health Association 1976).

PHYSICAL AND CHEMICAL PROCEDURES

Lighting

If a diel lighting regimen is followed, we suggest a 16-hr photoperiod and a lighting system similar to that described by Drummond and Dawson (1970). The system will eliminate sharp transitions between light and dark, more nearly approximating natural sunrise and sunset. Nevertheless, fish still react to the light-on and light-off periods with an increase in locomotor activity and opercular rate, and their opercular rate also tends to be higher during the day than it is at night, especially during the early morning hours. If desired, diel respiratory patterns (Sparks et al. 1972) can be nearly eliminated by using a continuous photoperiod. Another advantage of using continuous lighting is that a test can be started at any time of the day. For instance, imported effluent samples often arrive during the evening hours, and tests can be started immediately rather than allowing the effluent sample to age further.

Disturbances

If two or more chambers are constructed as a unit, an opaque shield may be needed between the chambers to prevent test fish from seeing one another. Although the bluegill sunfish in our cough tests were not influenced by the activities of their counterparts in adjacent chambers, other fish species may react differently. Also, all experimental fish must be well-shielded from visual and vibratory disturbances external to the test setup. Outside visual stimuli, such as a person walking by an unshielded test area, can greatly influence the fish's behavior. Typically, the fish will cease all movements, including respiratory, for a short time and then go through a period of increased locomotor activity and opercular rate. Opaque plastic curtains or styrofoam shields will effectively reduce or eliminate disturbances altogether.

Temperature, Oxygen, pH

Cairns, Morgan, and Sparks (1974) showed that bluegill sunfish breathing rates fluctuated with diurnal temperature changes, but they did not determine if cough rates also fluctuated with temperature change. Drummond (unpublished data) found that fluctuations in dissolved oxygen concentrations have a greater effect on opercular rate than on cough frequency. If the dissolved oxygen concentration is lowered, coughs will increase at about the same rate as opercular movements, i.e., if the opercular rate increases twofold, the cough rate will also. In contrast, fish exposed to a sublethal toxicant concentration in water with adequate dissolved oxygen may show a several-fold increase in cough rate without a corresponding increase in opercular rate. At lethal concentrations cough rate will increase about twice as fast as the opercular rate. Cough and opercular rates of some species may also be affected by extremes in pH (Hargis 1976). These variables should be monitored and controlled, but if they cannot be closely regulated then the degree of fluctuation should be specified and accounted for when analyzing test results.

Toxicant-Delivery System

Two methods of toxicant delivery gave consistently good results. Methods A and B shown in Figure 4 both deliver the toxicant solution to a cell where it is mixed with incoming dilution water. The desired mixture is then fed to a constant level headbox for further mixing and distribution to the electrode chambers. Method A requires a variable speed, multi-channel peristaltic pump, but is a versatile method because toxicant flow rates can be changed by simply altering pump speed.

Method B is a straightforward, inexpensive technique that works well when properly installed. The stock solution is maintained at a fairly constant level by periodically raising the screw-jack beneath the stock bottle. To maintain a steady toxicant flow rate, a large diameter bottle is required since the vertical loss of solution (and corresponding decrease in headpressure) is less in a wide bottle than it is in a narrow bottle over the same time span. The flow rate through the toxicant-delivery lines should be slow enough (0.1-2.0 ml/min) so that it will not decrease significantly overnight due to the decrease in headpressure. The rate at which the toxicant enters the mixing cell is therefore regulated by (1) the height of the stock bottle, (2) the level of the solution in the bottle, (3) the inside diameter of the Teflon tubing, and (4) an adjustable clamp attached to the Teflon delivery line. A mariotte bottle could be used instead, but we found flow rates to vary with fluctuations in room temperature, especially when solvent-toxicant stock solutions were required.

BIOLOGICAL PROCEDURES

Selection of Fish

In addition to those fish species mentioned in the review of literature, we have observed coughs in green sunfish (Lepomis cyanellus), largemouth bass

Figure 4. Dilution-water and toxicant-delivery systems.

DW - incoming dilution water

MHB - main headbox, large well-aerated reservoir

HB-1 - constant level distribution headbox (aeration optional); can provide flows to one or more mixing cells

MC - dilution water-toxicant mixing cell

HB-2 - constant level distribution headbox

SP - standpipe overflow drain

DT - hypodermic or capillary delivery tubes; provide equal flows to chambers

A - Method A: Toxicant delivery

SB - stock bottle

TG - Tygon tubing

PP - peristaltic pump

B - Method B: Toxicant delivery

SB - stock bottle

TF - Teflon tubing

AC - adjustable clamp (optional)

SJ - screw jack

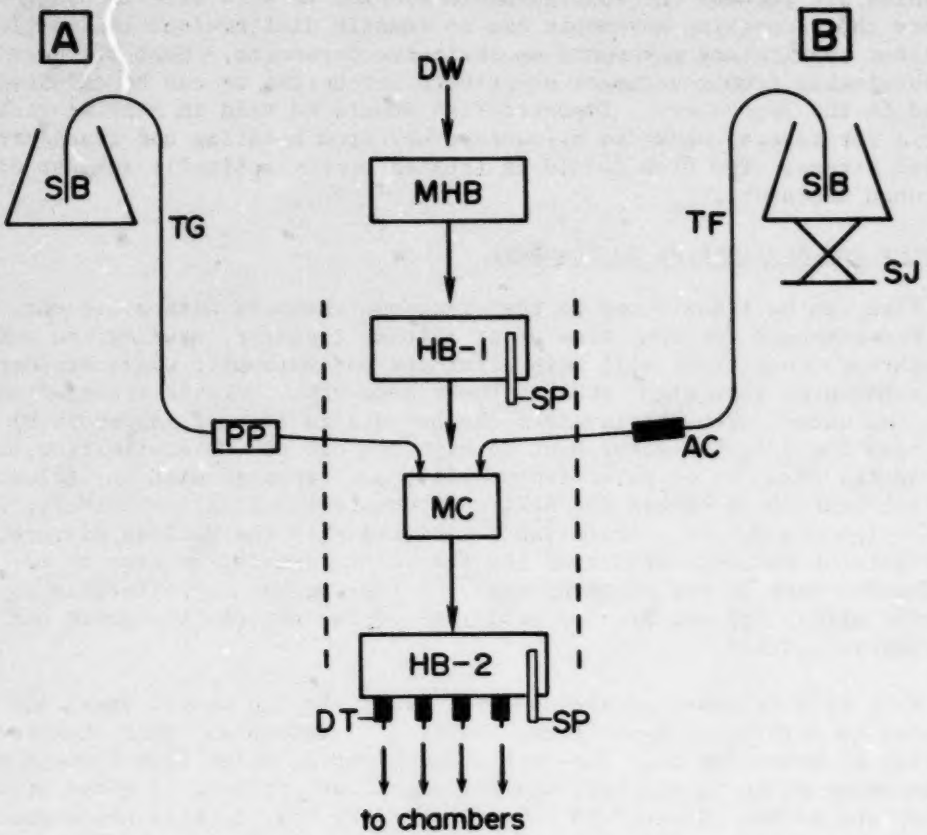


Figure 4. Dilution-water and toxicant-delivery systems.

(Micropterus salmoides), black crappie (Pomoxis nigromaculatus), northern pike (Esox lucius), and channel catfish (Ictalurus punctatus). Other species probably possess a cough mechanism also. We do not recommend a standard test fish, but suggest that priority be given to salmonids, bluegills, or green sunfish and, if test procedures can be worked out, the fathead minnow. Salmonids are perhaps the most amenable species to work with in cough tests because their coughing movements are so readily distinguishable from locomotor and other respiratory movements on strip-chart records. Most of these species are obtainable from government or private hatcheries or can be cultured and reared in the laboratory. Imported fish should be held in holding tanks or aquaria for several weeks to allow recovery from handling and transportation-induced stress. The fish should be treated prophylactically against disease only when necessary.

Transfer and Acclimation to Chambers

Fish can be transferred to the electrode chambers with a dip net. Since fish are stressed for some time after initial transfer, acclimation under flow-through conditions will help eliminate any metabolic waste products or fear substances that might inhibit their recovery. Further transfer is not required, except when the immersed chamber design is used (Appendix B). In this case the dilution water must be siphoned out of the acclimation tank, leaving the fish out of water temporarily, and replaced with an effluent or toxicant mixture to obtain the desired concentration. Alternatively, water can be siphoned out of a spare tank, replaced with the desired mixture, and the electrode chamber containing the test fish transferred from their acclimation tank to the prepared tank. Either method is preferable to another transfer with a dip net because handling-induced stress is reduced and recovery is quicker.

When airlift chambers are used for recirculating tests, stess due to transfer is eliminated by allowing the fish to remain in their chambers while the test is being set up. The fish are acclimated under flow-through conditions, the incoming water is stopped, and a toxicant or effluent is added after an appropriate amount of water is removed from the tray located below the chambers (Appendix A). Since water is removed only from the tray, the volume of water remaining in the electrode chambers must be included in calculations of test concentrations. Hence, effluent tests cannot be conducted in 100 percent effluent (by volume) unless the fish are transferred. This problem is apparently inconsequential, however, because test concentrations greater than 50 percent may not be necessary to assess the quality of an effluent (Carlson and Drummond 1977).

Acclimation time required for the fish to adjust to the electrode chambers is dependent to a certain extent on the fish species selected. Brook trout require 4 days, bluegill sunfish 3 days, and green sunfish 2 days to recover from the effects of transfer. The criterion we have used to determine proper acclimation is that time period required for cough and opercular rates and locomotor activity to decline to a base level, i.e., that point at which these activities do not vary significantly (P=0.05) from one day to the next. In no case has this period been less than 48 h.

Feeding is not recommended even though the acclimation and exposure period may last 7 days. If the fish are maintained in the chambers for longer periods, they should be fed once daily with a pelletized fish food. When fed, the chambers must be routinely cleaned to prevent buildup of feces and unused food, or the fish must be periodically transferred to clean chambers.

Preliminary Observations

Before starting cough tests, several fish should be individually observed to correlate visual observations of the different locomotor and respiratory activities with the strip-chart recordings. Indirect observation through one-way glass is preferable since fish often alter their behavioral patterns when observed directly. For instance, bluegills under moderate toxicant stress may stop coughing while an observer is present. Fish should be observed under control conditions as well as during exposure to a selected toxicant. Observe as many fish as possible at both sublethal and lethal toxicant concentrations while looking for characteristic cough patterns. When counting coughs, count all movements regardless of whether they appear to be weak or strong coughs (see review of literature). Some examples of cough movements recorded by a rectilinear polygraph are given in Appendix C. When coughs and other fish movements can be identified with confidence on the record sheets, visual observations can be discontinued.

Number of Test Fish

At sublethal concentrations intolerant fish may increase their cough rate greatly, whereas the more tolerant individuals may show little, if any, increase. If this variation in individual tolerance is not taken into account, the data may be biased and the test results invalidated. This variation may be minimized by using larger numbers of fish, but the number of fish tested at each concentration is largely dependent on the purpose of the test. If the purpose of the test is to detect spills of toxic materials in an on-site monitoring situation or to do laboratory screening of chemical substances or effluents (Carlson and Drummond 1977), then two fish per concentration may be all that is necessary. But if the intent is to rank chemicals according to their toxicity, as evidenced by increases in cough frequency, then at least four fish per concentration is a desirable number. A minimum of eight fish per concentration should be tested if the results are used to predict the concentration range at which long-term adverse effects are expected to occur (Drummond *et al.* 1973, 1974). Doubling the above numbers is biologically desirable, but will add considerably to the cost of the test.

Monitoring and Count Intervals

Frequency of the monitoring periods is dependent on the type of test conducted and the length of time required for cough frequency to reach a maximum. As a general rule, the more observations made, the better, since a large number (N) is desirable from the standpoint of statistical analyses.

Unless fish are being used to detect spills of toxic materials, each fish need not be monitored continuously. We suggest monitoring each fish at least once every 2 h during the 24-h period preceding the start of a test and for

the duration of the test. If previous experience shows that cough frequencies increase gradually over a period of days, the fish can be monitored less frequently, perhaps every 4-6 h. Each fish should be monitored for at least 15 min during each scheduled monitoring period.

A representative 5- or 10-min count interval should be selected from each 15-min monitoring period and the total number of coughs counted. A count interval should be selected that is smaller than the monitoring period for two reasons: first, it is too laborious to count all coughs over a 15-min period; and second, portions of the total record may be unreadable because swimming activity obscures cough and opercular movements. However, a 5- or 10-min count interval is necessary rather than a 1-min count interval because fish cough rates vary somewhat from minute to minute. These data are usually expressed as coughs per minute for use in statistically analyzing test results.

Length of Exposure

Generally, if fish do not show significant increases in cough rate within the first 96 h of exposure, they will probably not respond to prolonged exposure. An exception to this generality is fish exposed to the chlorinated hydrocarbons DDT and endrin (Schaumburg et al. 1967; Carlson, unpublished data). Fish exposed to these compounds show increases in cough rate that are time and concentration dependent. For instance, bluegills exposed to 6.0 µg/l endrin responded within 24 h with an increase in cough rate, but at 0.4 µg/l an increase was noted only after 240 h. Therefore, the exposure period may have to be extended for as long as 10 days to evaluate the effects on cough rates of certain chemical substances.

SECTION 8

INTERPRETATION AND APPLICATION OF COUGH-FREQUENCY DATA

STATISTICAL CONSIDERATIONS

Percentage change in cough frequency seems adequate to reflect stress in screening tests, but other tests require more elaborate treatment. Although there is no "standard" method for statistically analyzing cough-frequency data, and none is proposed at this time, we will outline those methods that have been used to judge the significance of an effect.

In using the electrode chamber method one can statistically compare a control group with an experimental group, or compare individual fish (or the same group of fish) before and after exposure. In tests with copper (Drummond et al. 1973) and mercury (Drummond et al. 1974), we compared the pretreatment cough frequencies to the frequencies on that day of exposure during which a group of fish, as a whole, showed a maximum increase. A paired t-test was used to test for significance of differences between cough frequencies before and during exposure (period of maximum increase). Walden et al. (1970) also used peak cough-frequency periods to evaluate the effect.

The selection of peak cough-frequency periods to evaluate an effect has the disadvantage of having to wait for the response to reach a maximum. To avoid this delay, we also have used Dunnett's procedure (Steel and Torrie 1960) for making a one-sided comparison between a treatment and a control mean. The effective concentrations were judged to be those at which at least one-half of the eight test fish showed significant (P, 95%) increases in cough frequency (Drummond, unpublished data). Other workers (McIntosh and Bishop 1976; Rice et al. 1977; Maki, personal communication) report using analysis of variance for analyzing cough and opercular data. Differences between means or log means were used in making these analyses.

Linear interpolation or extrapolation from a plotted line may be a better method for pinpointing no-effect concentrations. Walden et al. (1970) plotted the maximum and 5-h mean cough rates against effluent concentrations and extrapolated (best visual fit) to what they termed a "minimum response concentration." They indicated that linear plotting is a suitable technique for extrapolating to a minimum response concentration, provided the test concentrations are marginally above the level that initiates an increase in coughing. We agree that linear plots should be based on responses at test concentrations reasonably close to the no-effect level in that cough rates may become erratic at high concentrations.

PATTERN OF RESPONSE

The time at which peak cough frequencies occur for a group of fish and the pattern of decline after reaching a maximum may be important factors. Cough frequency in brook trout exposed to copper (Drummond et al. 1973) increased to a peak by the 20th hour of exposure. The pattern was the same for all test concentrations as schematically illustrated in Figure 5-A. When exposed to methylmercuric chloride (Drummond et al. 1974) the peak response was reached in 3 days (Figure 5-B). Fish exposed to chlorinated hydrocarbons (Schaumburg et al. 1967; Carlson, unpublished data) showed an entirely different pattern of response, as shown in Figure 5-C. The cough rate of trout exposed to neutralized kraft pulping effluents peaked during the second hour of exposure (Walden et al. 1970). These results suggested that the period of peak response may be diagnostic of the type of chemical substance to which the fish are exposed.

The response may, after reaching a peak, decline rapidly or slowly toward pretreatment levels under conditions of continuous exposure. The speed at which cough frequencies return to normal varies similarly when exposures are stopped at time of maximum response. Thus, the rate of decline may also be an important factor in classifying the type of chemical substance.

Although we have emphasized cough frequency because it is a more definitive response to sublethal concentrations than opercular rate and amplitude, various combinations of these respiratory responses, differences in their intensities, and differences in the rates at which they change may reflect the degree of effect that would be imposed upon the fish should toxicant exposure be prolonged. These responses can be categorized as follows:

Level I response: Cough frequency, opercular rate, and amplitude vary, but do not increase significantly from one day to the next. Response indicates normal expected variation for fish in ordinary water.

Level II response: Cough frequencies increase significantly without a corresponding increase in opercular rate or amplitude. Response indicates adverse long-term (chronic) effects.

Level III response: Large increases in cough frequency accompanied by moderate increases in opercular rate and amplitude. Response indicates sublethal to lethal effects.

Level IV response: Large and rapid increases in cough frequency, opercular rate, and amplitude. Response indicates lethal (acute) effects.

Level V response: Cough frequency, opercular rate, and amplitude greatly reduced or erratic after a level IV response. Response indicates that death is imminent.

Because these categories are founded on limited data, we have not attempted to quantify the break-points between them.

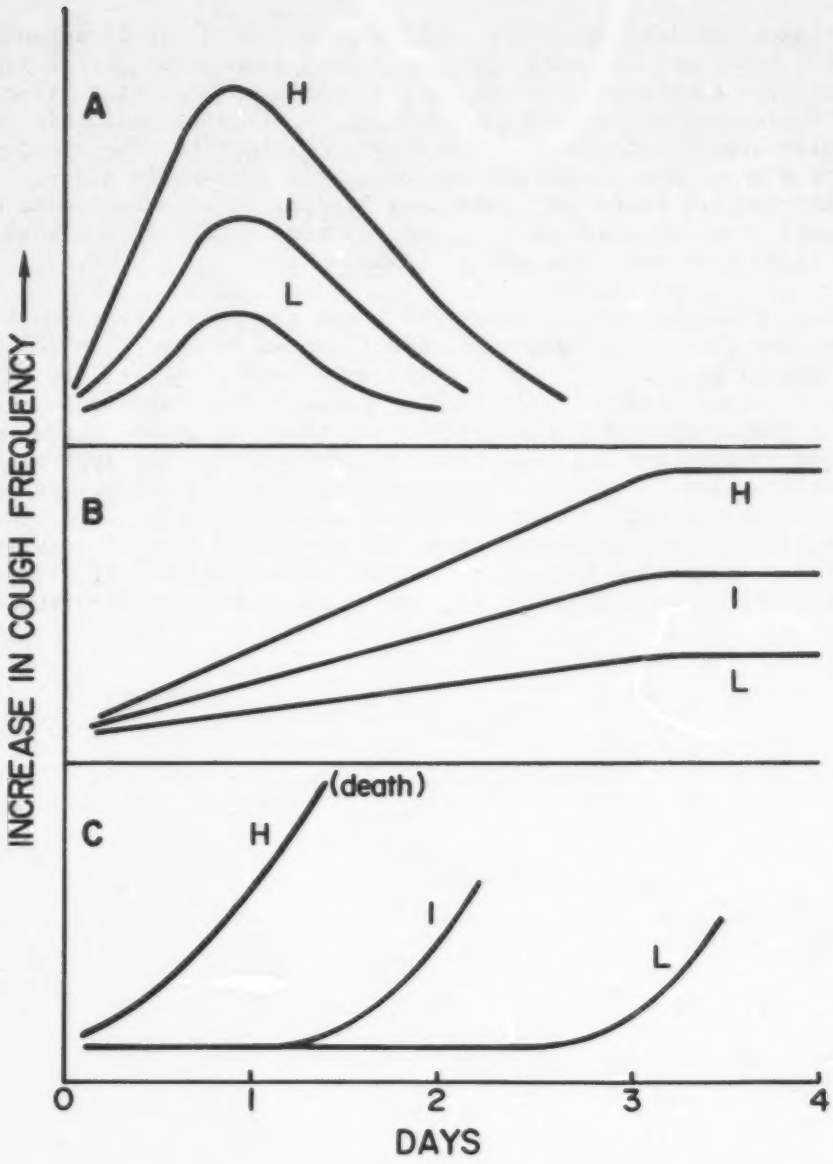


Figure 5. Cough-response patterns of brook trout exposed to copper (A), methylmercuric chloride (B), and chlorinated hydrocarbons (C). H, I, and L denote high, intermediate, and low concentrations.

UTILITY OF THE RESPONSE

The fish-cough test appears to have wide potential application as an endpoint for screening new chemicals for relative toxicity, predicting long-term effect concentrations, evaluating the quality of drinking water (Jung 1973) and surface water, and as an indicator of effluent toxicity. The latter is especially significant because the organism integrates the complex interrelationships of several possible toxicants present in a waste into a single, well-defined response. Sublethal biological effects cannot be predicted from physical-chemical measurements, nor can they be shown from 96-h acute mortality tests that only consider survival.

Of these possible applications, the cough response appears most useful as an endpoint for predicting long-term effect concentrations. In addition to copper (Drummond et al. 1973) and mercury (Drummond et al. 1974), we have found that other materials (Table 1) also cause significant increases in brook trout cough frequency near those MATC levels known to cause long-term effects such as increased mortality, reduction in growth rate, poor reproduction, and physical deformities. Although the present data base is small, information is sufficient to warrant further studies in which predicted effect concentrations can be verified by long-term exposures. If the cough test is consistently demonstrated to be a good indicator of MATC concentrations, it will also provide a short-term technique for evaluating the chronic toxicity of mixtures of toxicants.

TABLE 1. LOWEST CONCENTRATIONS OF TEN COMPOUNDS CAUSING SIGNIFICANT INCREASES IN BROOK TROUT COUGH FREQUENCY AND MATC RANGES FOR THIS SPECIES.

Compound	Effective concentration (µg/l)[1] Cough test	MATC range
Cadmium chloride	5	2 - 3[2]
Sodium dichromate	860	200 - 350[2]
Lead nitrate	80	58 - 119[2]
Zinc sulfate	1,392	532 - 1,368[2]
Copper sulfate	10	9 - 17[2]
Methylmercuric chloride	3	0.3 - 0.9[2]
Diazinon (Tech. grade 93.6%)	25	< 0.8[2]
Malathion (Tech. grade 95.0%)	7	< 16[3]
Lindane (ESA Pest. Ref. Standard 100%)	6	9 - 17[3]
DDT (pp' isomer 77.2%)	2	unavailable

[1]Measured concentration rounded to nearest whole number.

[2]McKim (1977).

[3]Andrew, R. W., et al. Evaluation of an application factor hypothesis. Envir. Res. Lab.-Duluth, unpublished manuscript.

REFERENCES

American Public Health Association. 1976. Standard methods for the examination of water and wastewater. 14th ed. 1193 p. New York.

Ballintijn, C. M., and G. M. Hughes. 1965. The muscular basis of the respiratory pumps in the trout. J. Exp. Biol. 43:349-362.

Ballintijn, C. M., and J. L. Roberts. 1976. Neural control and proprioceptive load matching in reflex respiratory movements of fish. Fed. Proc. 35:1983-1991.

Bass, M. L. 1975. A study of lethality and toxic mechanisms of intermittent chlorination to freshwater fish. Ph.D. Thesis. Virginia Polytechnic Institute and State University, Blacksburg, VA. 70 p.

Bert, P. 1870. Lecons sur la physiologie comparée de la respiration. J. -B. Balliére, Paris.

Bijtel, J. H. 1949. The structure and the mechanism of movement of the gill-filaments in teleostei. Arch. Néerl. Zool. 8:1-22.

Bimber, D. L., R. W. Boenig, and M. L. Sharma. 1976. Respiratory stress in yellow perch induced by subtoxic concentrations of diquat. Ohio J. Sci. 76:87-90.

Bull, C. J., and J. E. McInerney. 1974. Behavior of juvenile coho salmon (Oncorhynchus kisutch) exposed to sumithion (fenitrothion) an organophosphate insecticide. J. Fish. Res. Board Can. 31:1867-1872.

Cairns, J., Jr., J. W. Hall, E. L. Morgan, R. E. Sparks, W. T. Waller, and G. F. Westlake. 1973. The development of an automated biological monitoring system for water quality. Virginia Wat. Res. Center, Blacksburg, VA. Bull. 59. 50 p.

Cairns, J., Jr., E. L. Morgan, and R. E. Sparks, 1974. The response of bluegills (Lepomis macrochirus Rafinesque) in a pollution monitoring system to a diurnal temperature change. Trans. Am. Fish. Soc. 103:138-140.

Camougis, G. 1960. Recording bioelectric potentials from aquatic animals. Turtox News 38:156-157.

Carlson, R. W., and R. A. Drummond. 1977. Fish cough response - a method for evaluating quality of treated complex effluents. Water Res. (in press).

Dandy, J. W. T. 1967. Effects of chemical characteristics of the environment on the activity of an aquatic organism. Ph.D. Thesis. Univ. Toronto, Toronto, Ont. 263 p.

Dandy, J. W. T. 1972. Activity response to chlorine in brook trout, Salvelinus fontinalis (Mitchill). Can. J. Zool. 50:405-410.

Davis, J. C. 1973. Sublethal effects of bleached kraft pulp mill effluent on respiration and circulation in sockeye salmon (Oncorhynchus nerka). J. Fish. Res. Board Can. 30:369-377.

Davis, J. C. 1976. Progress in sublethal effect studies with kraft pulpmill effluent and salmonids. J. Fish. Res. Board Can. 33:2031-2035.

Dessy, R. E. 1976. Microprocessors - an end user's view. Science. 192:511-518.

Drummond, R. A. 1977. Disposable electrode chamber for measuring opercular movements of fathead minnows. Prog. Fish-Cult. 39:94.

Drummond, R. A., and W. F. Dawson. 1970. An inexpensive method for simulating diel patterns or lighting in the laboratory. Trans. Am. Fish. Soc. 99:434-435.

Drummond, R. A., and W. F. Dawson. 1974. An inexpensive solidstate amplifier for detecting movements and electrical potentials of fish. Trans. Am. Fish. Soc. 103:391-392.

Drummond, R. A., G. F. Olson, and A. R. Batterman. 1974. Cough response and uptake of mercury by brook trout (Salvelinus fontinalis) exposed to mercuric compounds at different hydrogen-ion concentrations. Trans. Am. Fish. Soc. 103:244-249.

Drummond, R. A., W. A. Spoor, and G. F. Olson. 1973. Some short-term indicators of sublethal effects of copper on brook trout, Salvelinus fontinalis. J. Fish. Res. Board Can. 30:698-701.

Ewing, G. W., and H. A. Ashworth. 1974. The laboratory recorder. Plenum Press, New York. 129 p.

Ferris, C. D. 1974. Introduction to bioelectrodes. Plenum Press, New York. 243 p.

Francois-Franck, C. A. 1906. Analyse graphique de movements respiratoires des poissons teleosteens. Compt. Rend. Seances Soc. Biol. Filiales 60:799-802.

Goodman, D. A., and N. M. Weinberger. 1971. Submerged electrodes in an aquarium: validation of a technique for remote sensing of behavior. Behav. Res. Meth. Instr. 3:281-286.

Hargis, J. R. 1976. Ventilation and metabolic rate of young rainbow trout (Salmo gairdneri) exposed to sublethal environmental pH. J. Exp. Zool. 196:39-44.

Heath, A. G. 1972. Critical comparison of methods for measuring fish respiratory movements. Water Res. 6:1-7.

Heimstra, N. W., D. K. Damkot, and N. G. Benson. 1969. Some effects of silt turbidity on behavior of juvenile largemouth bass and green sunfish. U.S. Fish Wildl. Serv., Bur. Sport Fish. Wildl. Tech. Pap. 20.

Holeton, G. F., and D. R. Jones. 1975. Water flow dynamics in the respiratory tract of the carp (Cyprinus carpio L.). J. Exp. Biol. 63:537-549.

Howard, T. E., and C. C. Walden. 1974. Measuring stress in fish exposed to pulp-mill effluents. Tappi. 57:133-135.

Hughes, G. M. 1960. A comparative study of gill ventilation in marine teleosts. J. Exp. Biol. 37:28-45.

Hughes, G. M. 1973. Respiratory responses to hypoxia in fish. Am. Zool. 13:475-489.

Hughes, G. M. 1975. Coughing in the rainbow trout (Salmo gairdneri) and the influence of pollutants. Rev. Suisse Zool. 82:47-64.

Hughes, G. M., and R. J. Adeney. 1977. Variations in the pattern of coughing in rainbow trout. J. Exp. Biol. 68:109-122.

Hughes, G. M., and M. Morgan. 1973. The structure of fish gills in relation to their respiratory function. Biol Rev. 48:419-475.

Hughes, G. M., and J. L. Roberts. 1970. A study of the effect of temperature changes on the respiratory pumps of the rainbow trout. J. Exp. Biol. 52-177-192.

Hughes, G. M., and R. L. Saunders. 1970. Response of the respiratory pumps to hypoxia in rainbow trout (Salmo gairdneri). J. Exp. Biol. 53:529-545.

Hughes, G. M., and G. Shelton. 1958. The mechanism of gill ventilation in three freshwater teletostean fishes. J. Exp. Biol. 35:807-823.

Jung, K. D. 1973. Substances extremely toxic to fish and their importance for a fish test warning system. GWF-Wasser/Abwasser. 114:232-234.

Kuiper, T. 1907. Untersuchungen Über die Atmung der Teleostei. PflÜgers. Arch. Ges. Physiol. 177:1-107.

Lunn, C. R., D. P. Toews, and D. J. R. Pree. 1976. Effects of three pesticides on respiration, coughing, and heart rates of rainbow trout (Salmo gairdneri Richardson). Can. J. Zool. 54:214-219.

MacLeod, J. C., and L. L. Smith, Jr. 1966. Effect of pulpwood fiber on oxygen consumption and swimming endurance of the fathead minnow, Pimephales promelas. Trans. Am. Fish. Soc. 95:71-84.

Martens, D. W., R. W. Gordon, and J. A. Servizi. 1970. Toxicity of copper to sockeye and pink salmon during their freshwater life. Int. Pac. Salmon Fish. Comm., Manuscript Report, 27 p.

McCutcheon, F. H. 1970. Stimulation, control and phylogenetic projection of the teleostean yawn reflex. Comp. Biochem. Physiol. 34:399-344.

McIntosh, A., and W. Bishop. 1976. Distribution and effects of heavy metals in a contaminated lake. Water Resour. Res. Center, Purdue Univ., Tech. Rep. 85. 69 p.

McKim, J. M. 1977. Evaluation of tests with early life stages of fish for predicting long-term toxicity. J. Fish. Res. Board Can. 34:1148-1154.

McKim, J. M., and D. A. Benoit. 1971. Effects of long-term exposures to copper on survival, growth, and reproduction of brook trout (Salvelinus fontinalis). J. Fish. Res. Board Can. 28:655-662.

McKim, J. M., G. F. Olson, G. W. Holcombe, and E. P. Hunt. 1976. Long-term effects of methylmercuric chloride on three generations of brook trout (Salvelinus fontinalis): toxicity, accumulation, distribution, and elimination. J. Fish. Res. Board Can. 33:2726-2739.

McPherson, B. P. 1973. Effects of methylmercury exposure on sea water adaptation of juvenile salmonids. M.S. Thesis. Oregon State Univ., Corvallis, Ore. 89 p.

Morgan, W. S. G. 1976. Fishing for toxicity: biological auto-monitor for continuous water quality control. Effl. Water Treat. 16:471-475.

Morgan, W. S. G. 1977. Biomonitoring with fish: an aid to industrial effluent and surface water quality control. Prog. Water Tech. (Great Britain) 9:703-711.

Morgan, W. S. G., and P. C. Kuhn. 1974. A method to monitor the effects of toxicants upon breathing rate of largemouth bass (Micropterus salmoides Lacepede). Water Res. 8:67-77.

Mount, D. I., and C. E. Stephan. 1967. A method for establishing acceptable toxicant limits for fish--malathion and the butoxyethanol ester of 2,4-D. Trans. Am. Fish. Soc. 96:185-193.

Rice, S. D., R. E. Thomas, and J. W. Short. 1977. Effect of petroleum hydrocarbons on breathing and cough rates and hydrocarbon uptake-depuration in pink salmon fry. In: Physiological Responses of Marine Biota to Pollutants. Academic Press, Inc., New York. p. 259-277.

Rommel, S. A., Jr. 1973. A simple method for recording fish heart and operculum beats without the use of implanted electrodes. J. Fish. Res. Board Can. 30:693-694.

Rovainen, C. M. 1977. Neural control of ventilation in the lamprey. Federation Proc. 36:2386-2389.

Satchell, G. H., and D. J. Maddalena. 1972. The cough or expulsion reflex in the Port Jackson shark, Heterodontus portus jacksoni. Comp. Biochem. Phys. 41A:42-62.

Saunders, R. L. 1961. The irrigation of the gills in fishes. I. Studies of the mechanism of branchial irrigation. Can. J. Zool. 39:637-653.

Schaumburg, F. D., T. E. Howard, and C. C. Walden. 1967. A method to evaluate the effects of water pollutants on fish respiration. Water Res. 1:731-737.

Sellers, C. M., Jr., A. G. Heath, and M. L. Bass. 1975. The effect of sublethal concentrations of copper and zinc on ventilatory activity, blood oxygen and pH in rainbow trout (Salmo gairdneri). Water Res. 9:401-408.

Servizi, J. A., R. W. Gordon, and D. W. Martens. 1969. Marine disposal of sediments from Bellingham Harbour as related to sockeye and pink salmon fisheries. Int. Pac. Salmon Fish. Comm. Prog. Rep. 23. 38 p.

Shelton, G. 1959. The respiratory center in the tench (Tinca tinca). I. The effects of brain transection on respiration. J. Exp. Biol. 36:191-202.

Skidmore, J. F. 1970. Respiration and osmoregulation in rainbow trout with gills damaged by zinc sulphate. J. Exp. Biol. 52:481-494.

Sparks, R. E., J. Cairns, Jr., and A. G. Heath. 1972. The use of bluegill breathing rates to detect zinc. Water. Res. 6:895-911.

Sparks, R. E., J. Cairns, Jr., R. A. McNabb, and G. Sutter, II. 1972. Monitoring zinc concentrations in water using the respiratory response of bluegills (Lepomis macrochirus Rafinesque). Hydrobiologia 40:361-369.

Spoor, W. A., T. W. Neiheisel, and R. A. Drummond. 1971. An electrode chamber for recording respiratory and other movements of free-swimming animals. Trans. Am. Fish. Soc. 100:22-28.

Sprague, J. B. 1964. Avoidance of copper-zinc solutions by young salmon in the laboratory. J. Water Poll. Cont. Fed. 36:990-1004.

Sprague, J. B. 1971. Measurement of pollutant toxicity to fish. III. Sublethal effects and "safe" concentrations. Water Res. 5:245-266.

Steel, R. G. D., and J. H. Torrie. 1960. Principles and procedures of statistics. McGraw-Hill Co., Inc. New York. 481 p.

Strange, J. R., J. W. Dean, G. K. Dorman, and D. J. Fletcher. 1975. New method for recording heart and respiratory rates in catfish. Sci. Biol. 1:17-18.

U. S. Environmental Protection Agency. Committee on Methods for Toxicity Tests with Aquatic Organisms. 1975. Methods for acute toxicity tests with fish, macroinvertebrates, and amphibians. Rep. EPA-660/3-75-009. Nat. Tech. Inf. Ser., U.S. Dep. Comm., Springfield, VA. 61 p.

Venables, G., and E. N. Smith. 1972. A simple method for recording opercular rate and heart rate of unrestrained fish. Prog. Fish-Cult. 34:233-234.

Walden, C. C., T. E. Howard, and G. C. Froud. 1970. A quantitative assay of the minimum concentration of kraft mill effluents which affect fish respiration. Water Res. 4:61-68.

Young, S. 1970. EMG activity in tench (Tinca tinca) gill lamellae and its association with coughing. J. Physiol. (London) 215:37-38.

Young, S. 1972. Electromyographic activity during respiration and coughing in tench (Tinca tinca L.). J. Physiol. (London) 227:18-19.

APPENDICES

Appendix A. An air-lift chamber system designed for effluent testing. System also can be used for testing single toxicants.

Each chamber (15 cm long x 4 cm wide x 10.5 cm high) exposes fish to effluent or control water air-lifted up glass tubes from an effluent tray into the chamber (Figure A-1). One electrode is glued to the drain end, and the other electrode is mounted on a 3-cm x 10-cm moveable glass baffle. The baffle is suspended in the chamber by exiting the electrode on one side and a short stainless steel wire hanger on the other, both resting in notches cut in the top of each side. Three sets of notches, at 6 cm, 9 cm, and 12 cm from the drain end, permit chamber size to be adjusted to accommodate any fish 3-10 cm long. A 2-cm square of plexiglas glued to the rear upper part of the moveable baffle prevents the fish from pushing the electrode toward the water inlet when a glass lid is in place and weighted. The 4-cm x 13-cm lid covers the chamber except for the water inlet. A sliding 6-mm-diameter glass standpipe adjusts chamber volume to 250 for a centrarchid or 125 ml for small salmonids.

Four individual chambers are placed in a three-sided box constructed so that the drain end of each chamber extends beyond the bottom on the open side (Figure A-1). The box is made of 1-cm plywood coated with fiberglass and rests on the sides of a water bath. Each water bath consists of a 5 1/2-gal aquarium containing an effluent tray in place of the four-plex, immersed chamber described in Appendix B. The effluent tray (23 cm long x 18 cm wide x 13 cm high) is divided into two equal compartments, and the bottom edge of a drain hole drilled in one end for each half of the tray is exactly 10 cm off the tray bottom. Volume of each compartment is 2,000 $\pm$ 50 ml when filled to the drain hole and supplies water or effluent to two individual electrode chambers.

Each lift tube is a 23-cm length of 8-mm glass tubing connected with rubber tubing to one side of a 1/4-in. Kimax glass connecting u-tube. The constriction is cut off the u-tube to make it flush with the lift tube, and the unused common opening of the u-tube is plugged with glass glue. The other side of the u-tube extends over the front of the plywood box into the electrode chamber water inlet (Figure A-1). Air is supplied through 3-mm glass tubing bent 180 degrees at one end to fit into the bottom of the lift tube. Air flow is regulated with three-way valves, and when properly adjusted a maximum lift of 65 ml/min is possible. Flows are maintained at approximately 50 ml/min through each chamber during testing, and the chambers drain directly back into the effluent tray. During recirculating tests the drain hole is stoppered, and the water-delivery tubes are flipped back out of the effluent trays. The dilution water then flows into the surrounding water baths to maintain effluents and fish at test temperature.

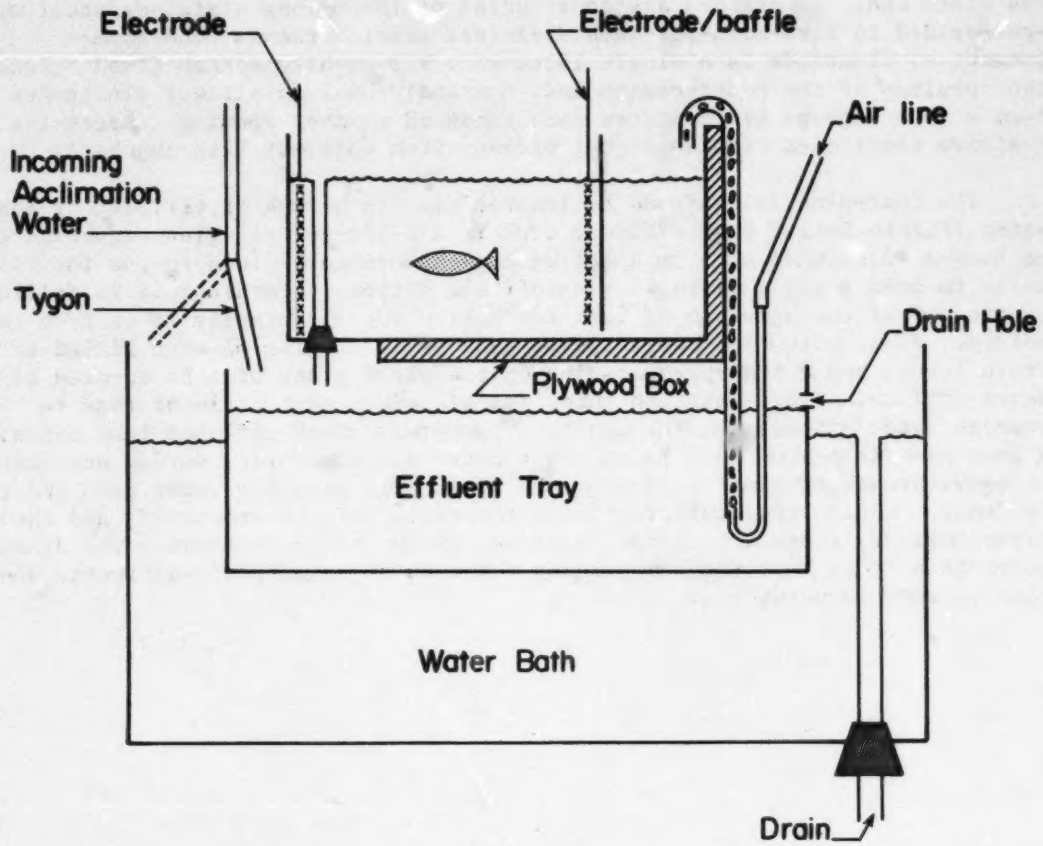
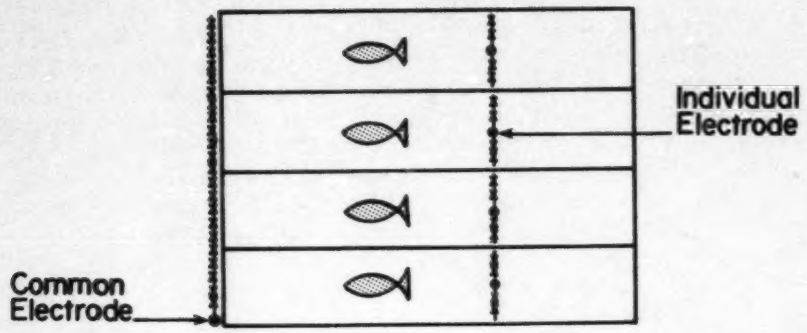


Figure A-1. Airlift chamber system.

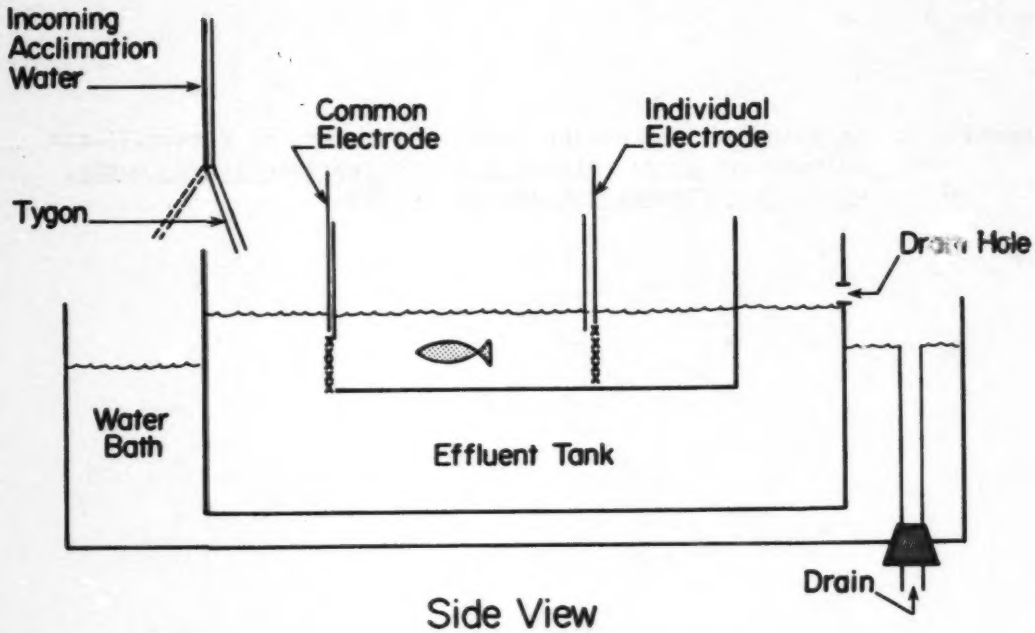
Appendix B. A four-plex, immersed chamber system designed for effluent testing. System also can be used for testing single toxicants.

Each four-plex consists of a series of glass partitions 18 cm long x 10 cm high and sides mounted on a base plate 18 cm long x 15 cm wide (Figure B-1). End pieces (3 cm wide x 7 cm high) glued 3 cm off the base separate the partitions and sides and are mounted flush with one end, but recess 7 cm from the other end. Electrodes are constructed of 18-cm-long stainless steel wires spot-welded to five-mesh-per-inch stainless steel screens. The common (negative) electrode is a single 15-cm-wide x 3-cm-high screen glued across the openings of the non-recessed end, and individual (positive) electrodes are 3-cm x 4-cm screens glued across each recessed chamber opening. Recessing the positive electrodes reduces signal pick-up from adjacent fish chambers.

The four-plex is designed to immerse fish in a tank of effluent or control water (Figure b-1). Each effluent tank is a 5-1/2-gal all-glass aquarium (40 cm long x 20 cm wide x 25 cm high) with glass brackets glued to the interior walls to hold a glass plate 11.5 cm off the bottom. A drain hole is drilled in one end of the aquarium so that its bottom edge is exactly 18 cm from the bottom. Final volume of the effluent tank is 14 l $\pm$ 100 ml when filled to drain level, and a four-plex resting on the glass plate will be covered to a depth of 7 cm. Glass aeration tubes are placed in each effluent tank to promote water circulation through the four-plex. Each effluent tank containing a four-plex is placed in a large water bath, and water flow during acclimation or flow-through testing is through the drain hole into the water bath and then to drain. During recirculating tests the drain hole is stoppered, and the water-delivery tubes are flipped back out of the effluent tanks. The dilution water then flows into the surrounding water baths to maintain effluents and fish at test temperatures.



Top View (chamber only)



Side View

Figure B-1. Immersed chamber system.

Appendix C. Examples of fish coughs (arrows) recorded by a rectilinear polygraph. Note: Arrows point to representative coughs, others are present but are not marked.

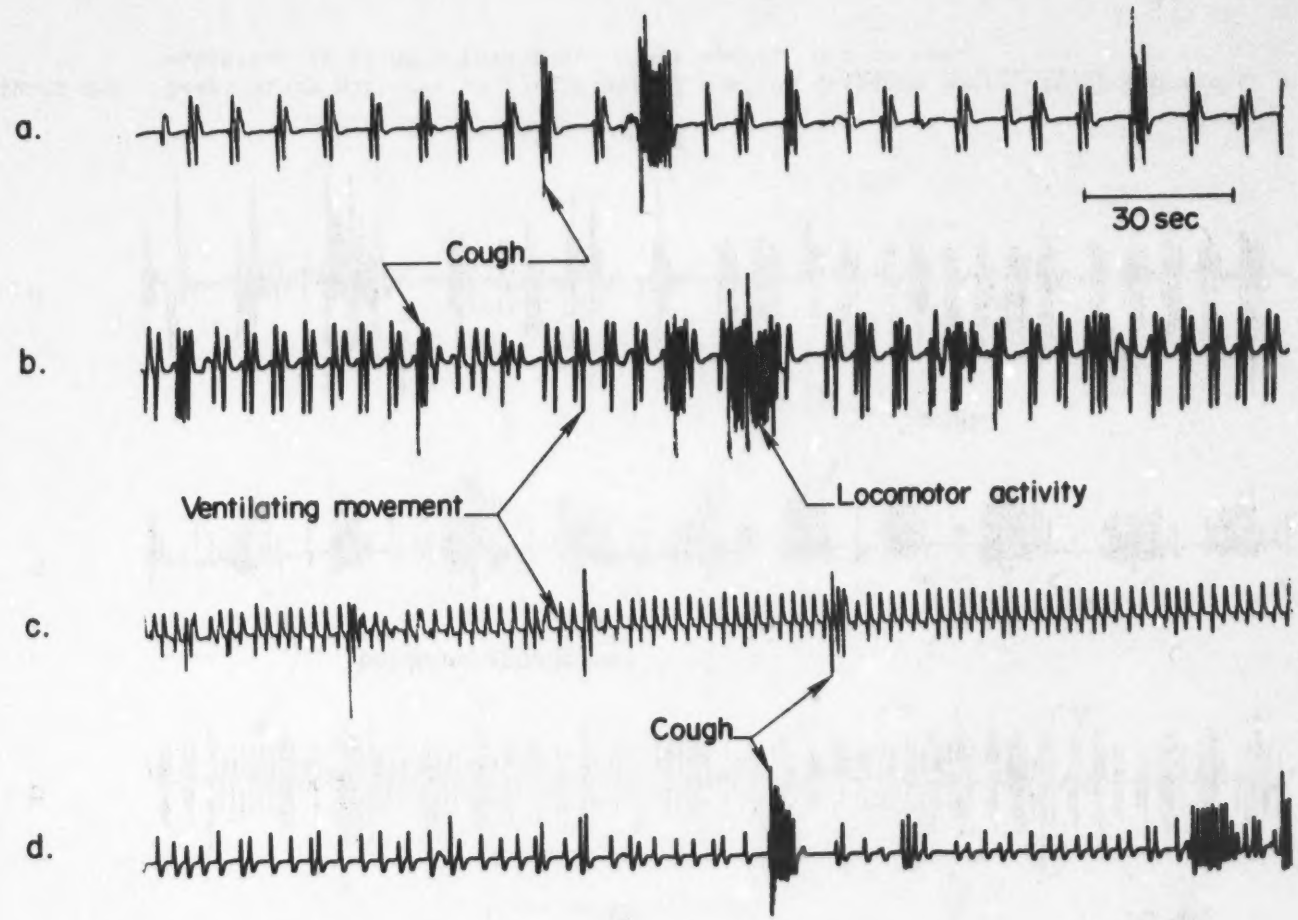


Figure C-1. Respiratory patterns of four bluegill sunfish (Lepomis macrochirus) in control water. Chart speed: 0.1 cm/sec.

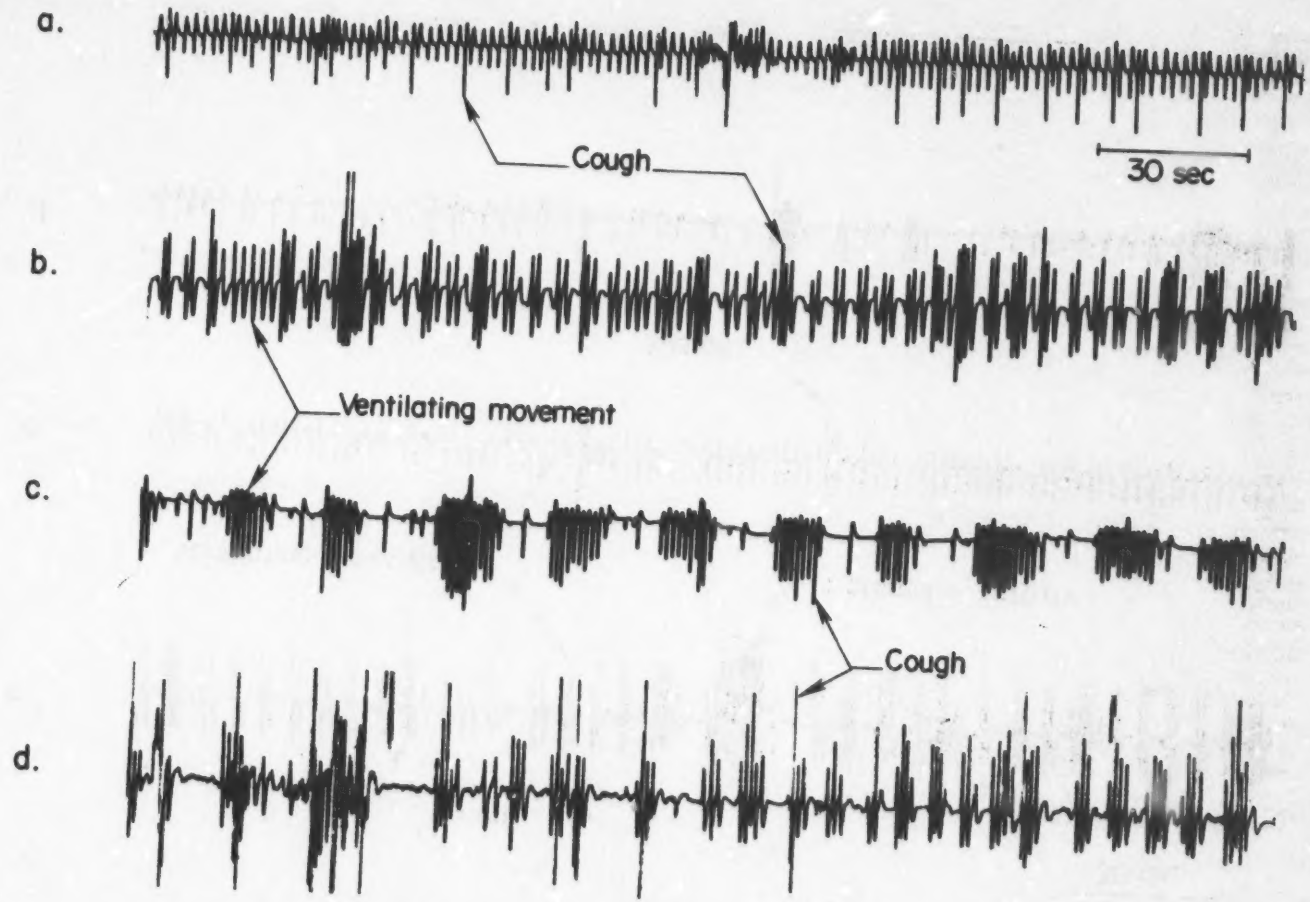


Figure C-2. Respiratory patterns of four bluegill sunfish (Lepomis macrochirus) exposed to a sublethal level of a toxicant. Chart speed: 0.1 cm/sec.

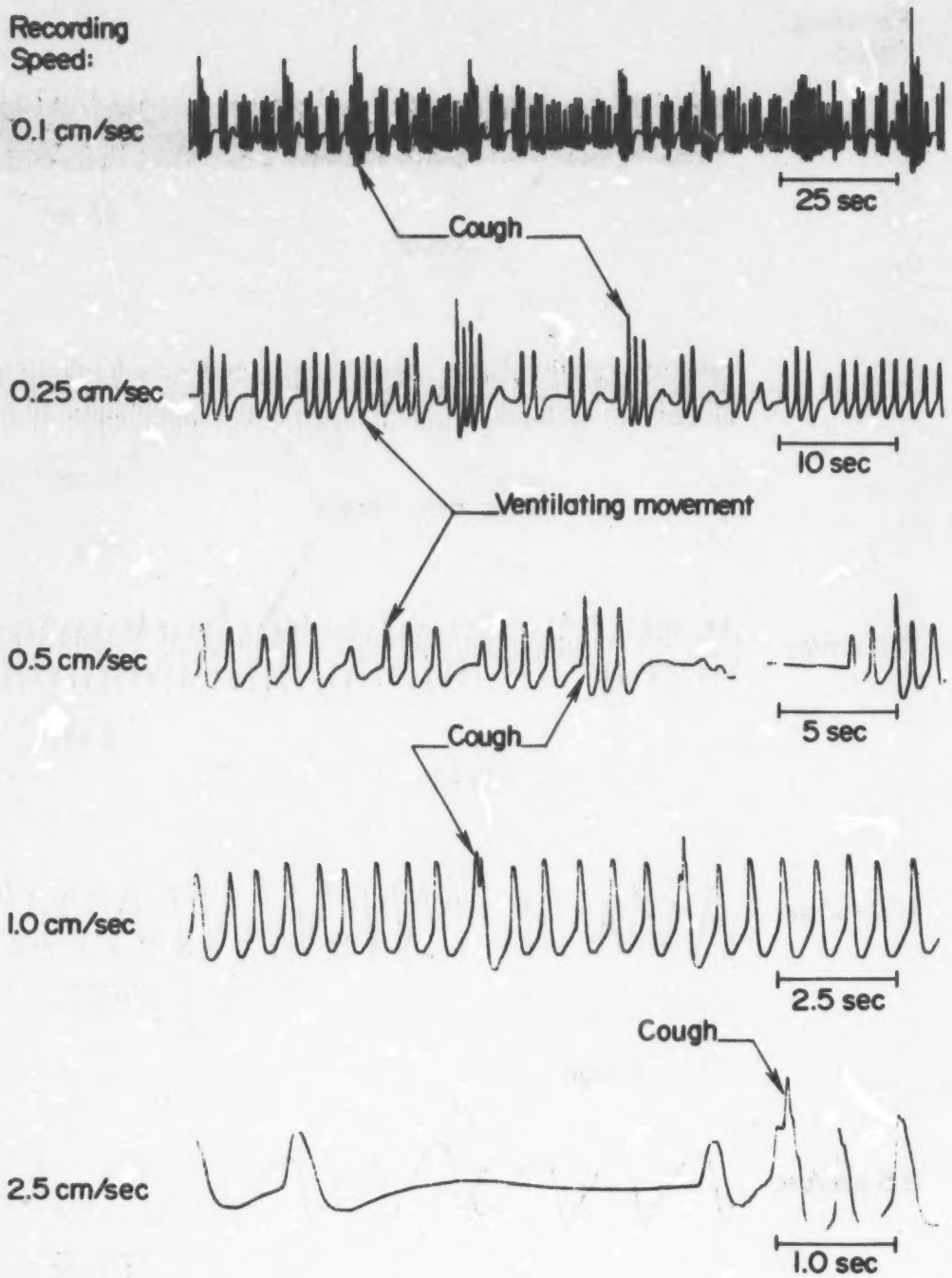


Figure C-3. Respiratory pattern of bluegill sunfish (Lepomis macrochirus) at 72-hr exposure to a sublethal level of zinc. Strip-chart records are of the same fish, but at different paper speeds.

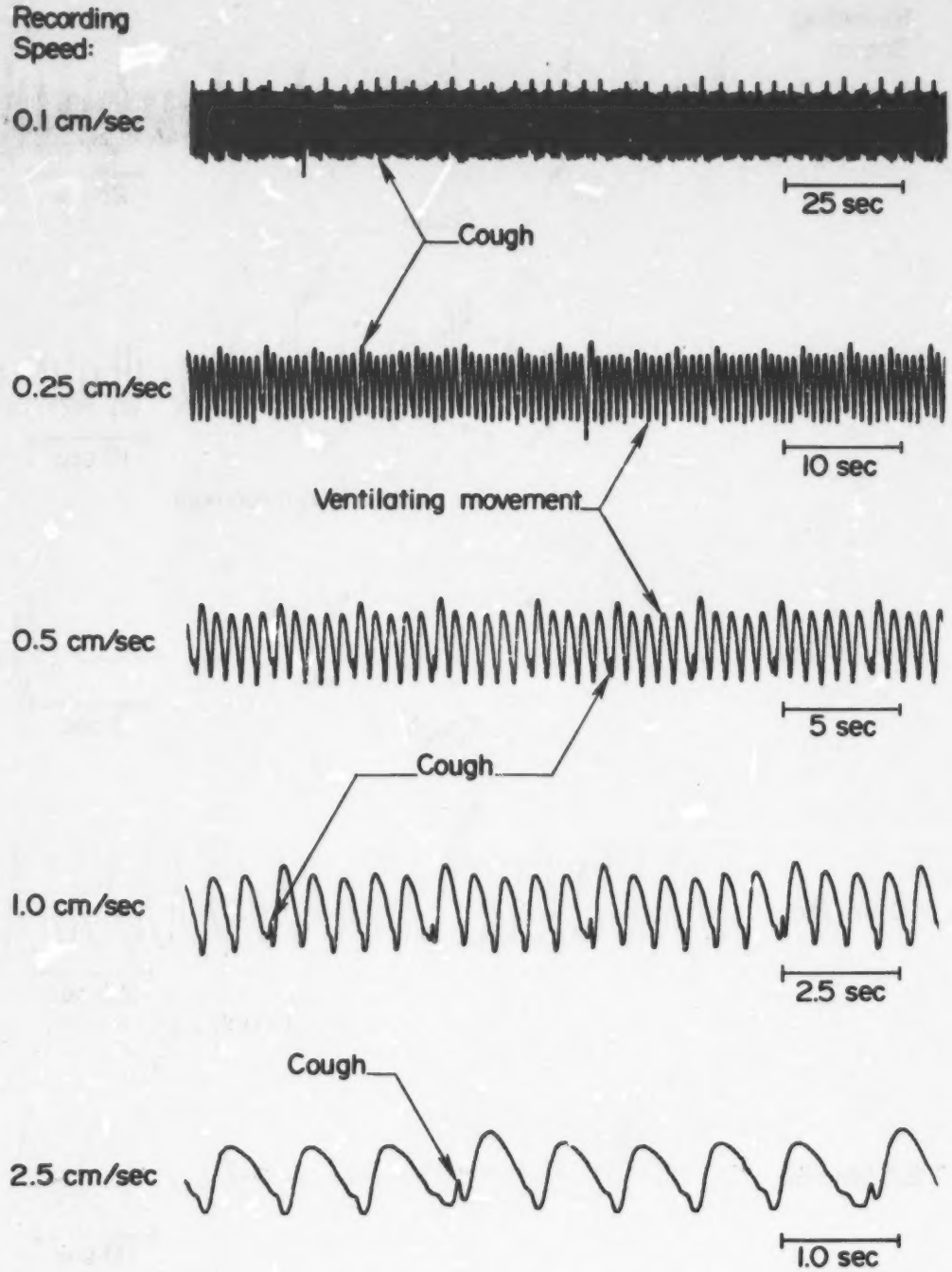


Figure C-4. Respiratory pattern of bluegill sunfish (Lepomis macrochirus) at 24-hr exposure to a lethal level of zinc. Strip-chart records are of the same fish, but at different paper speeds.

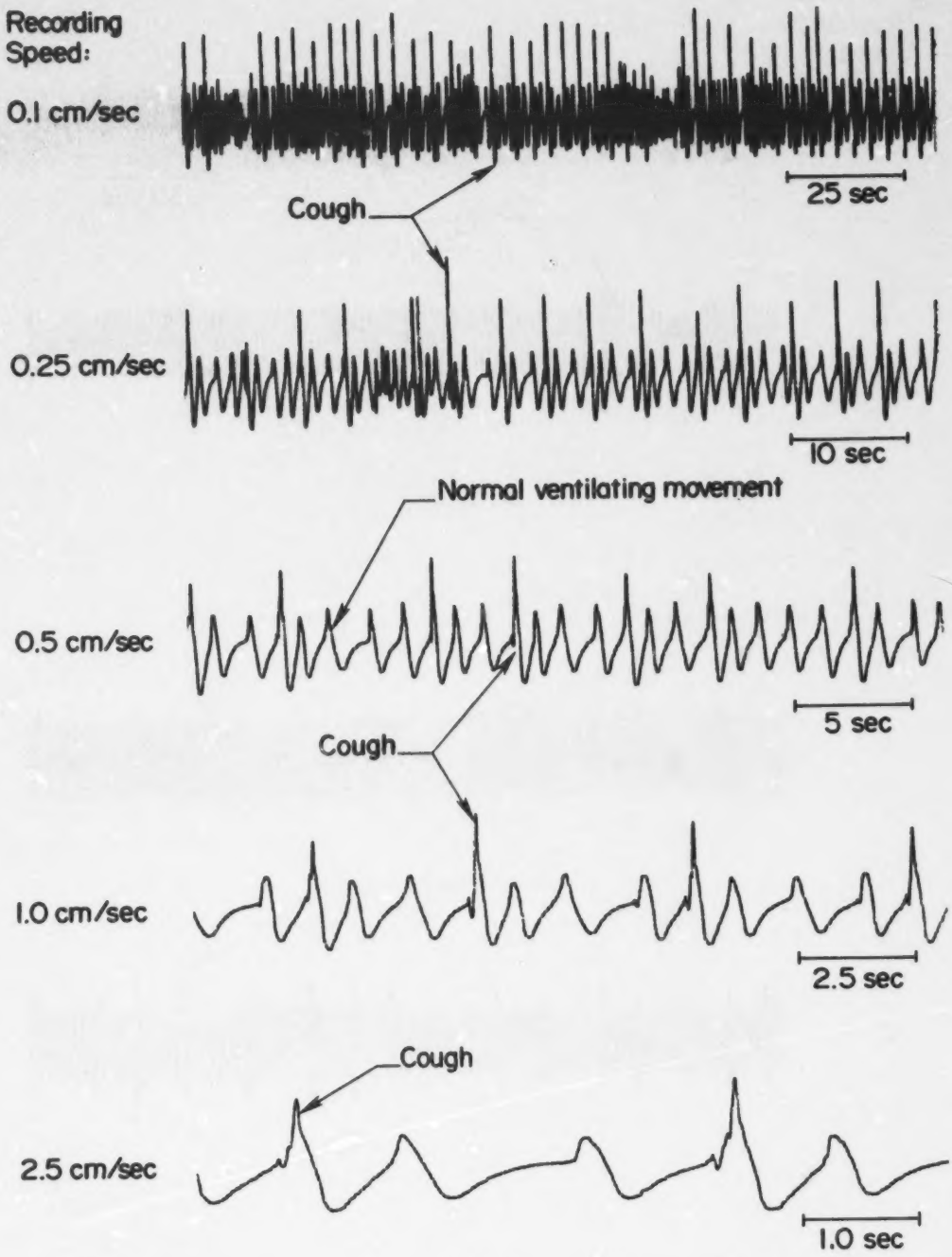


Figure C-5. Respiratory pattern of bluegill sunfish (Lepomis macrochirus) at 30-hr exposure to a lethal level of pentachlorophenol. Strip-chart recordings are of the same fish, but at different paper speeds.

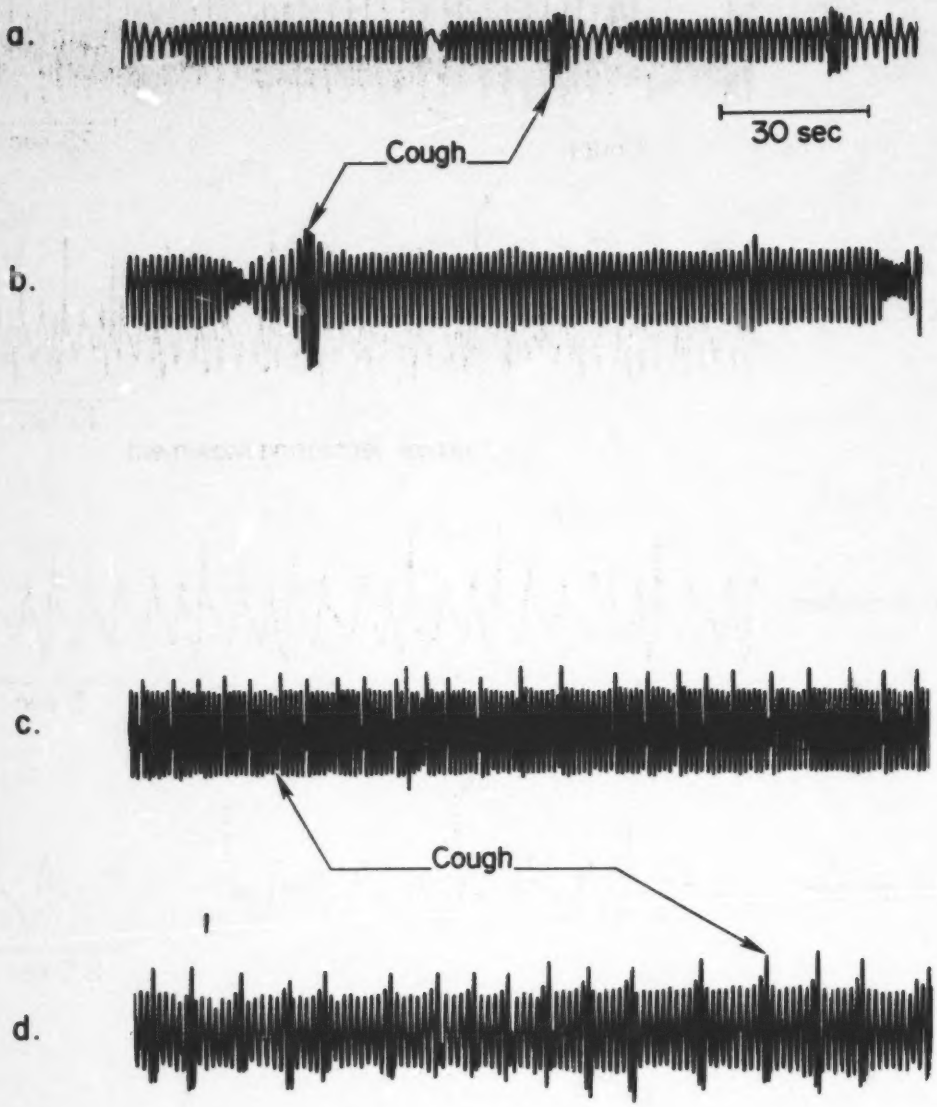


Figure C-6. Respiratory patterns of brook trout (Salvelinus fontinalis) while a,b) in control water, and c,d) undergoing toxicant stress. Chart speed was 0.1 cm/sec.